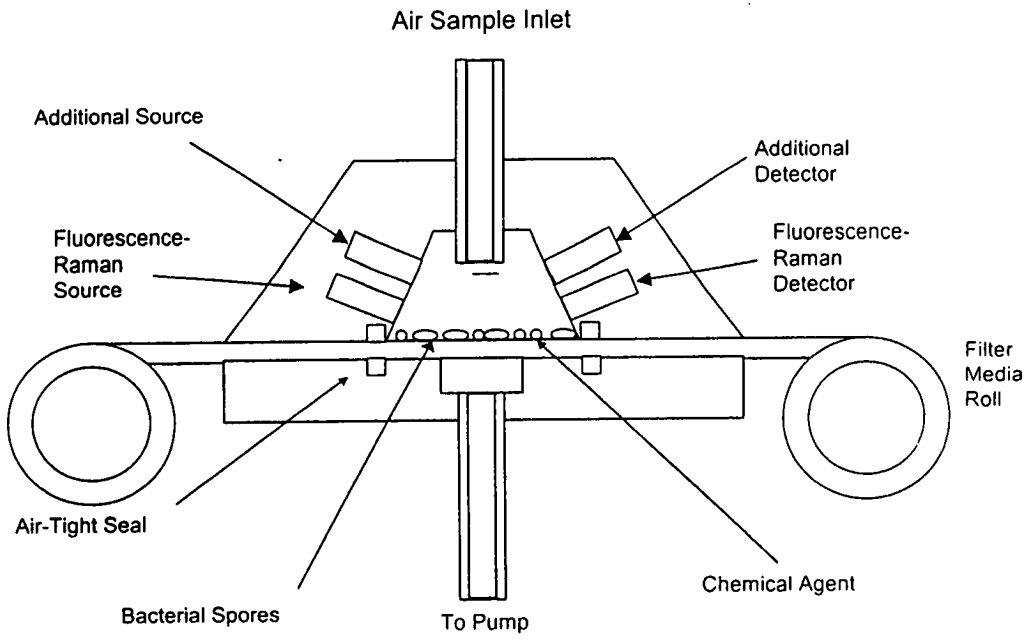
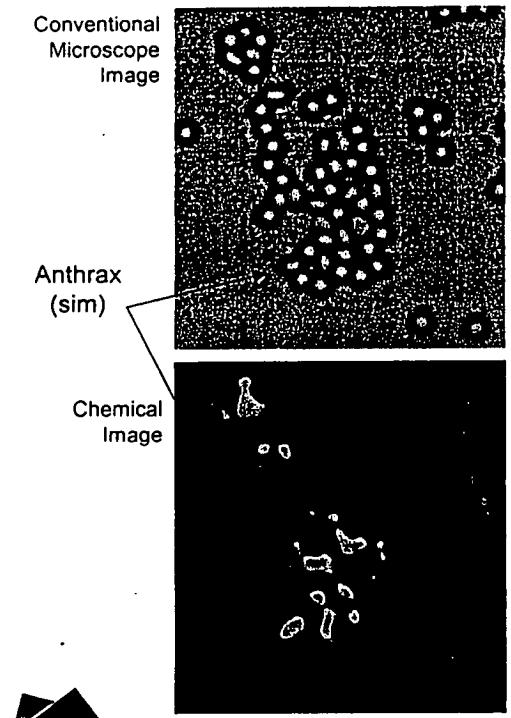


The Integrated Detector Sample Cell

Figure 1

Raman Chemical Imaging of Anthrax Simulant

Using a ChemIcon FALCON™ Raman Chemical Imaging Microscope



- Optical diagnostic technology with potential for rapid anthrax identification
- Single bacterium detection limit
- Applicable to other biological and chemical threats
- Laboratory technology commercially available today
- Resources needed to convert laboratory technology to field portable technology and accelerate production

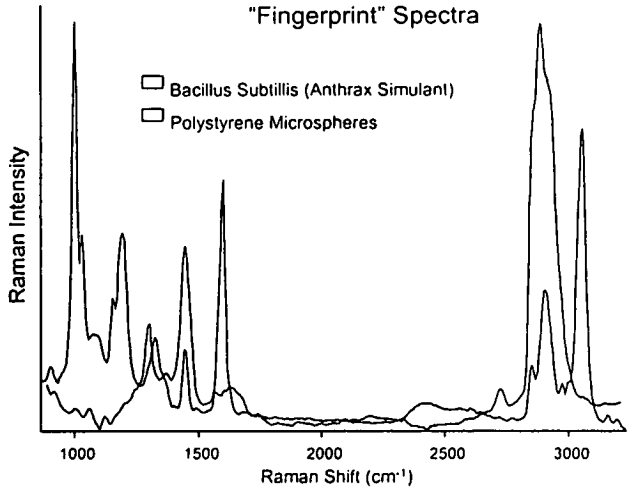


Figure 2

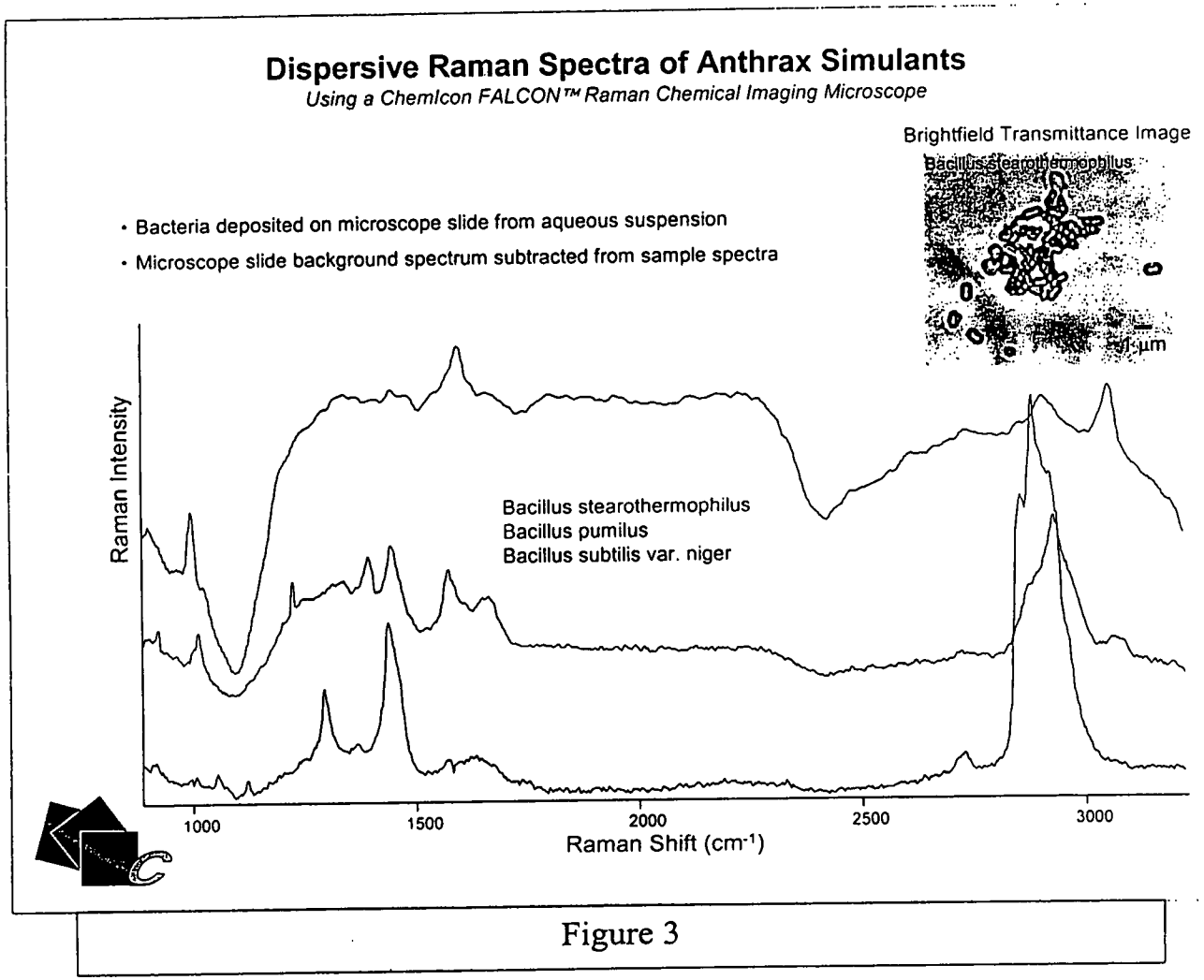

Dispersive Raman Spectra of Anthrax Simulants
Using a ChemIcon FALCON™ Raman Chemical Imaging Microscope
Brightfield Transmittance Image
Bacillus stearothermophilus
1 µm
• Bacteria deposited on microscope slide from aqueous suspension
• Microscope slide background spectrum subtracted from sample spectra
Raman Intensity
Bacillus stearothermophilus
Bacillus pumilus
Bacillus subtilis var. niger
1000
1500
2000
2500
3000
Raman Shift (cm-1)

Figure 3

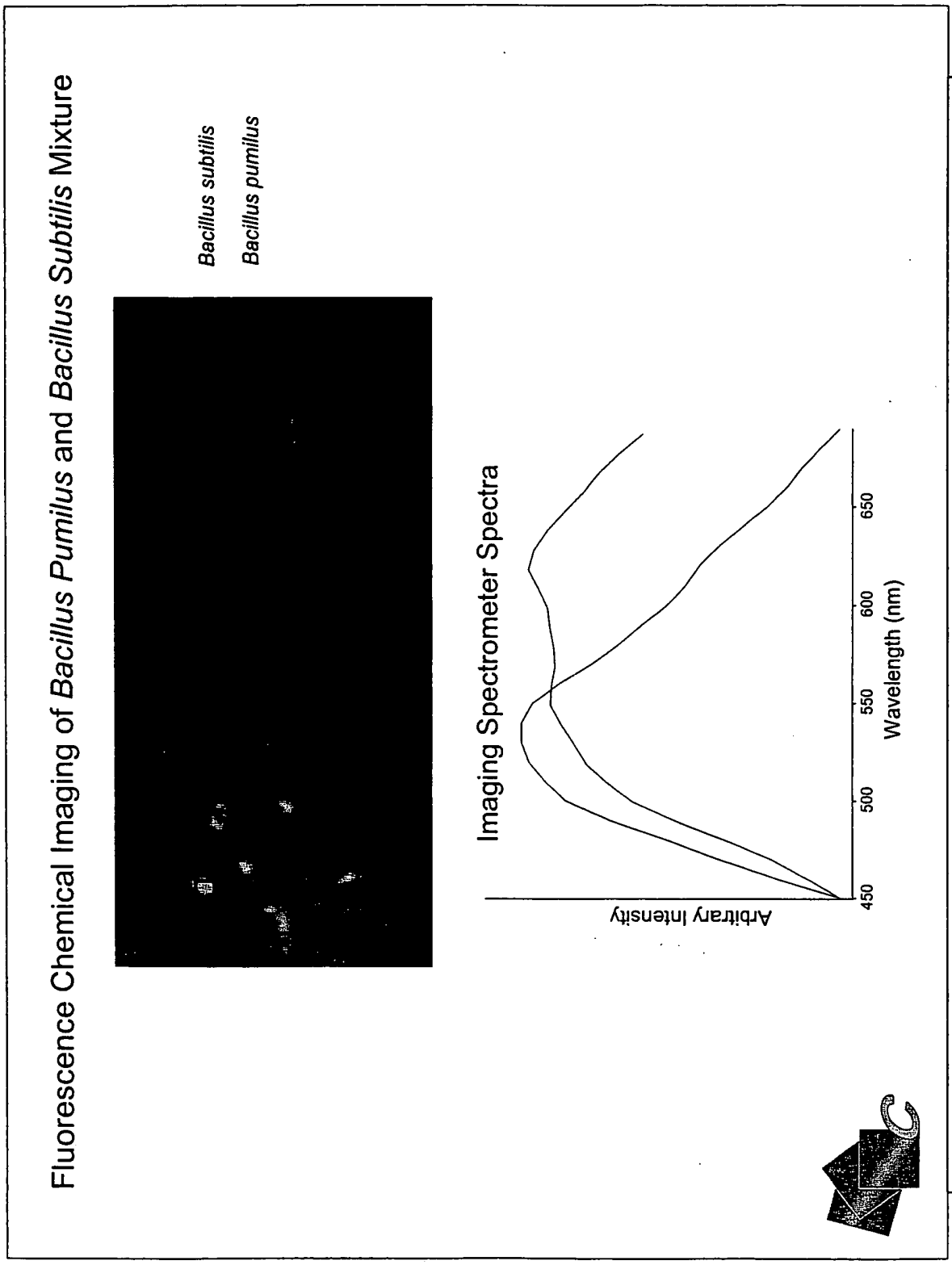

Fluorescence Chemical Imaging of Bacillus Pumilus and Bacillus Subtilis Mixture
Bacillus subtilis
Bacillus pumilus
Imaging Spectrometer Spectra
Arbitrary Intensity
450
500
550
600
650
Wavelength (nm)

Figure 4

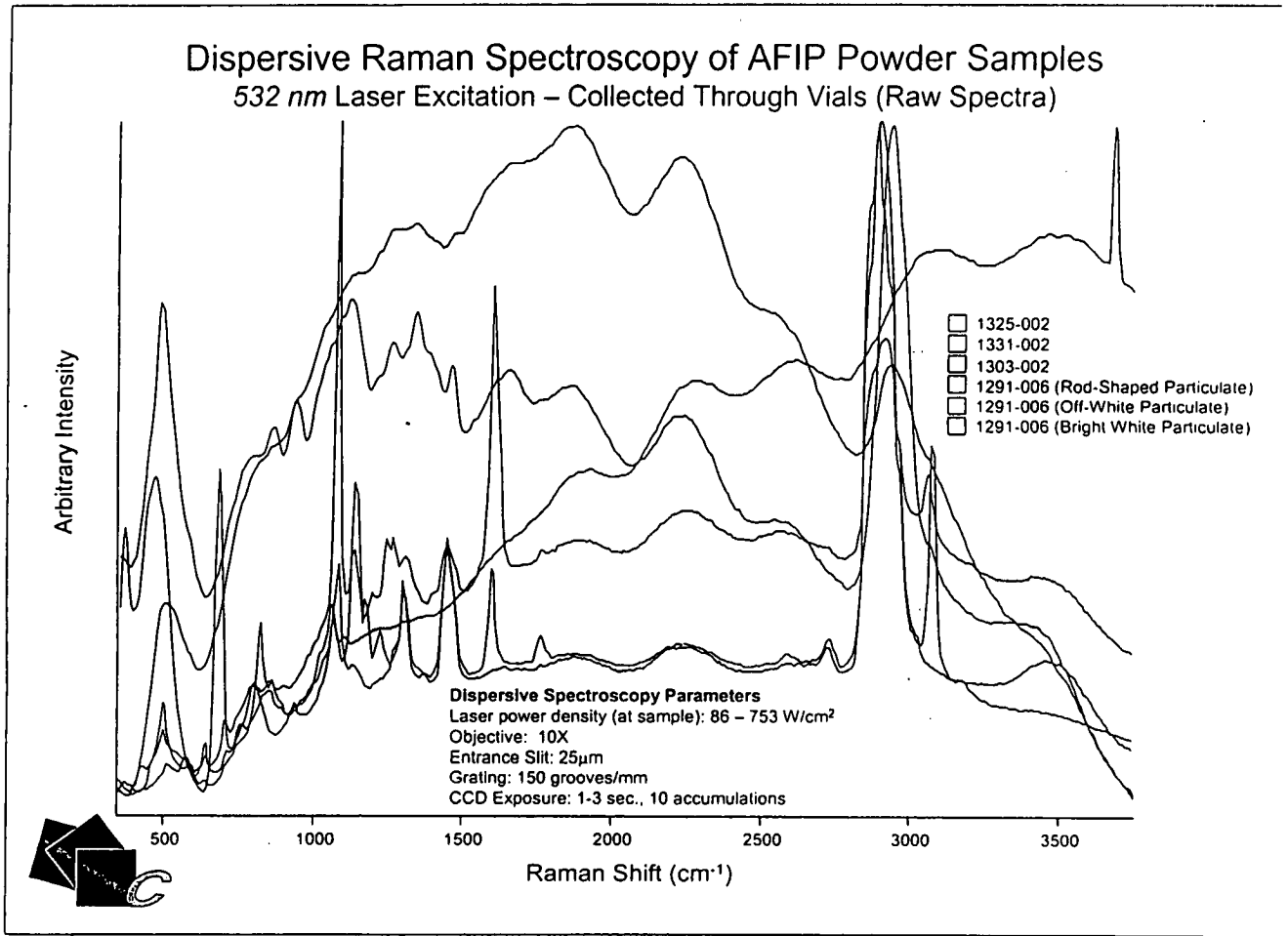

Dispersive Raman Spectroscopy of AFIP Powder Samples
532 nm Laser Excitation – Collected Through Vials (Raw Spectra)
Arbitrary Intensity
1325-002
1331-002
1303-002
1291-006 (Rod-Shaped Particulate)
1291-006 (Off-White Particulate)
1291-006 (Bright White Particulate)
Dispersive Spectroscopy Parameters
Laser power density (at sample): 86 – 753 W/cm²
Objective: 10X
Entrance Slit: 25µm
Grating: 150 grooves/mm
CCD Exposure: 1-3 sec., 10 accumulations
500
1000
1500
2000
2500
3000
3500
Raman Shift (cm-1)

Figure 5A

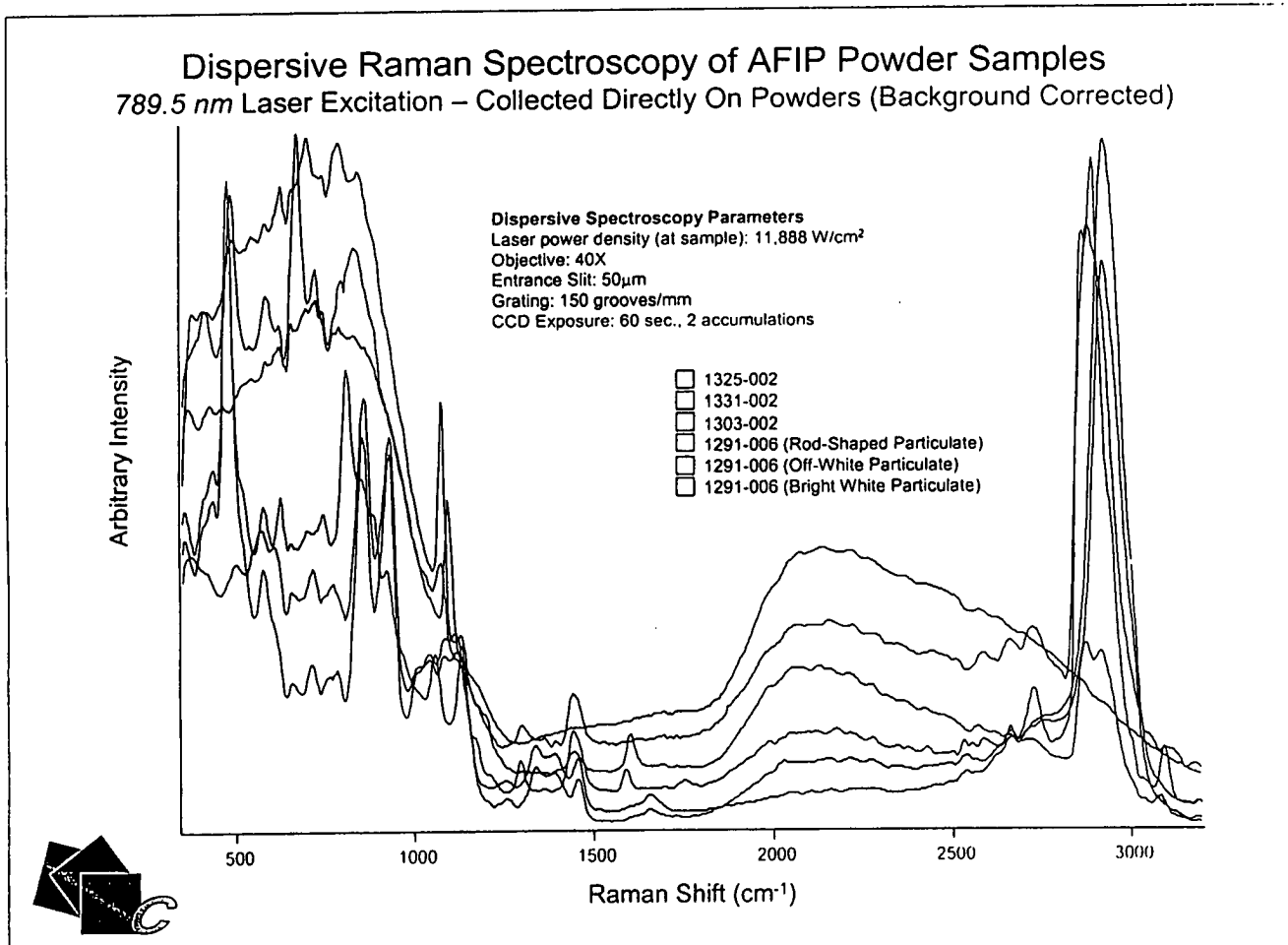

Dispersive Raman Spectroscopy of AFIP Powder Samples
789.5 nm Laser Excitation – Collected Directly On Powders (Background Corrected)
Dispersive Spectroscopy Parameters
Laser power density (at sample): 11,888 W/cm²
Objective: 40X
Entrance Slit: 50µm
Grating: 150 grooves/mm
CCD Exposure: 60 sec., 2 accumulations
1325-002
1331-002
1303-002
1291-006 (Rod-Shaped Particulate)
1291-006 (Off-White Particulate)
1291-006 (Bright White Particulate)
Arbitrary Intensity
500
1000
1500
2000
2500
3000
Raman Shift (cm-1)

Figure 5B

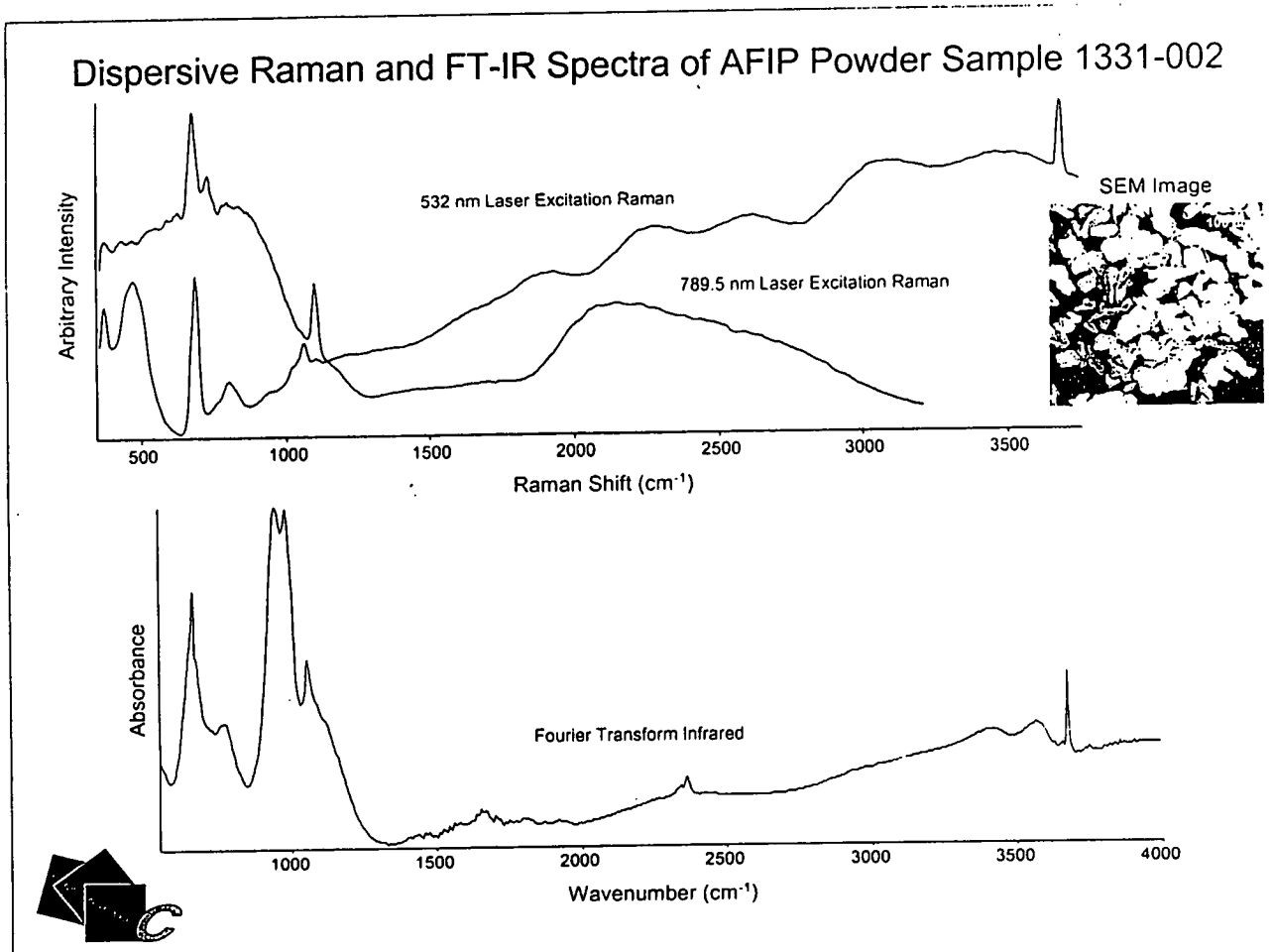

Dispersive Raman and FT-IR Spectra of AFIP Powder Sample 1331-002
Arbitrary Intensity
532 nm Laser Excitation Raman
789.5 nm Laser Excitation Raman
SEM Image
500
1000
1500
2000
2500
3000
3500
Raman Shift (cm-1)
Absorbance
Fourier Transform Infrared
1000
1500
2000
2500
3000
3500
4000
Wavenumber (cm-1)
C

Figure 5C

SEM/EDS of AFIP Powder Sample 1331-002

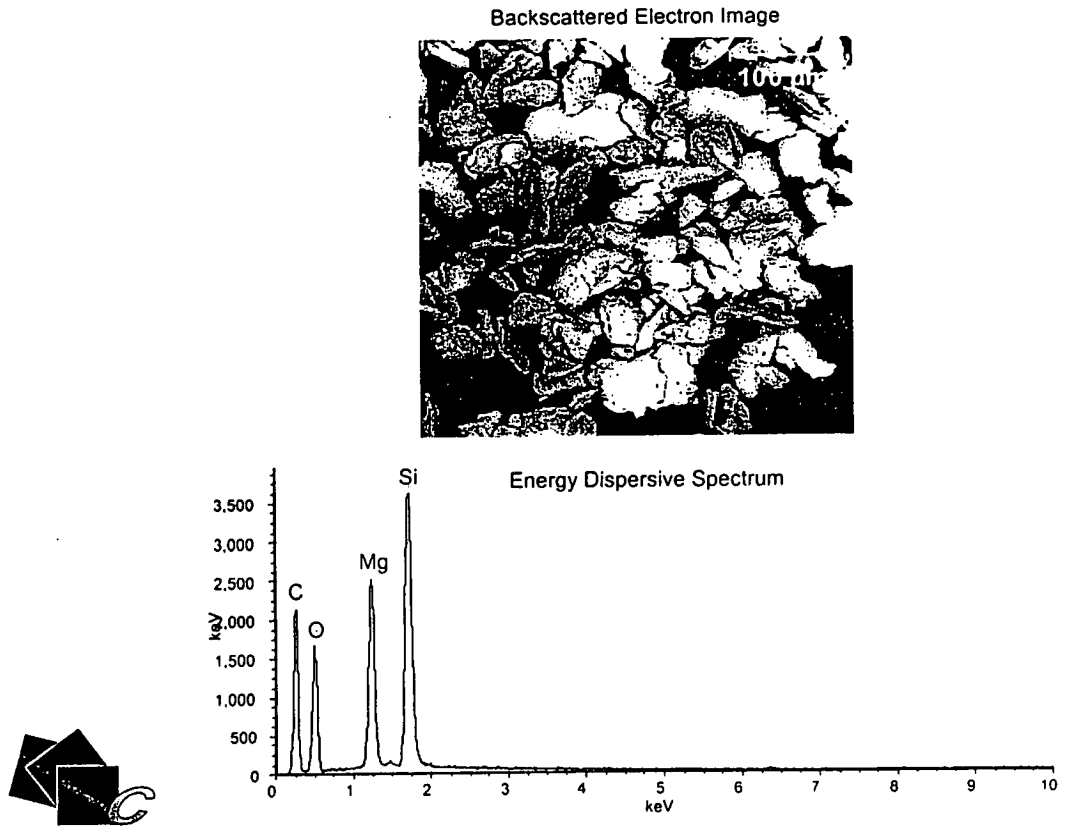


Figure 5D

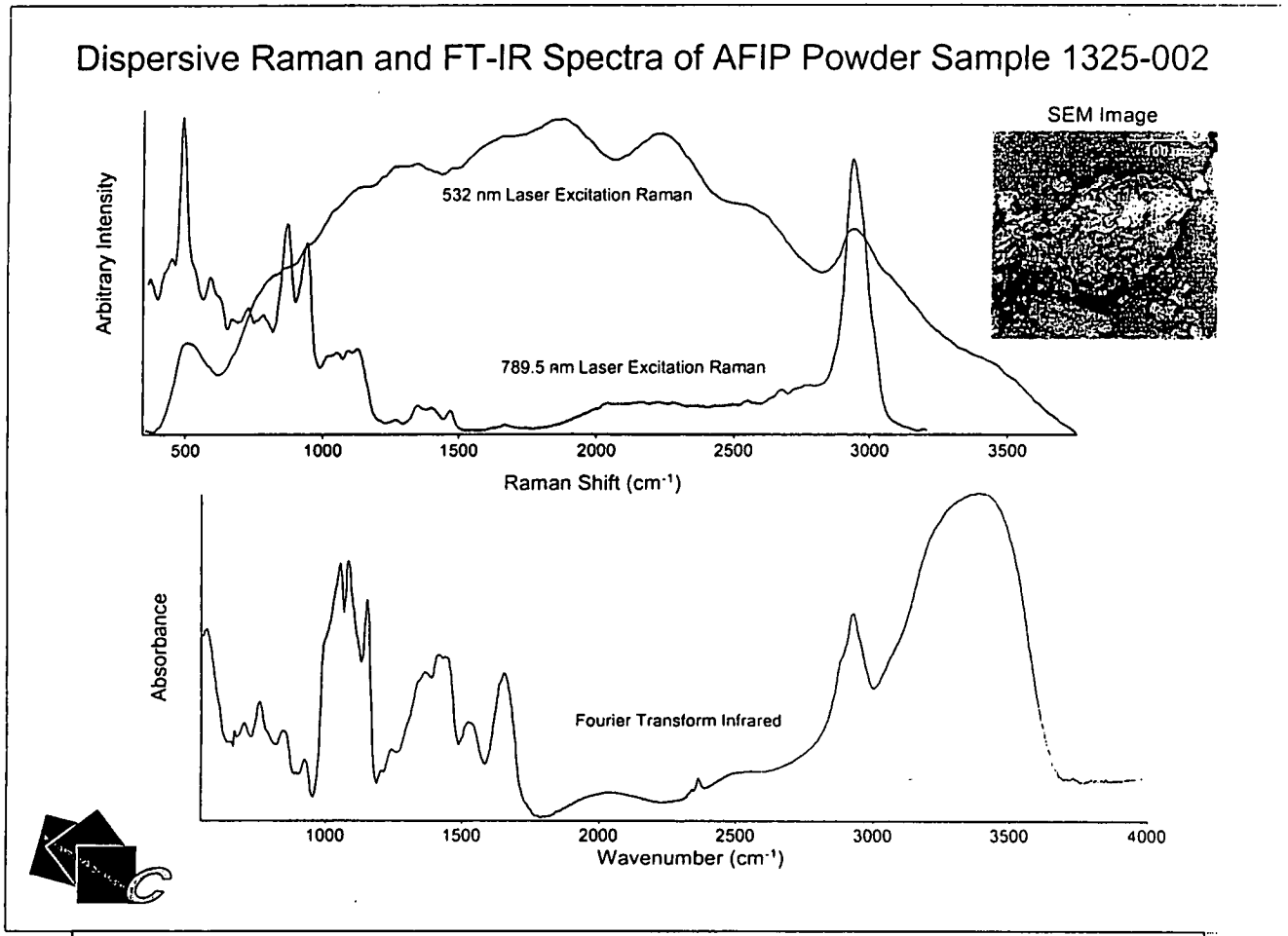

Dispersive Raman and FT-IR Spectra of AFIP Powder Sample 1325-002
SEM Image
Arbitrary Intensity
532 nm Laser Excitation Raman
789.5 nm Laser Excitation Raman
500
1000
1500
2000
2500
3000
3500
Raman Shift (cm-1)
Absorbance
Fourier Transform Infrared
1000
1500
2000
2500
3000
3500
4000
Wavenumber (cm-1)

Figure 5E

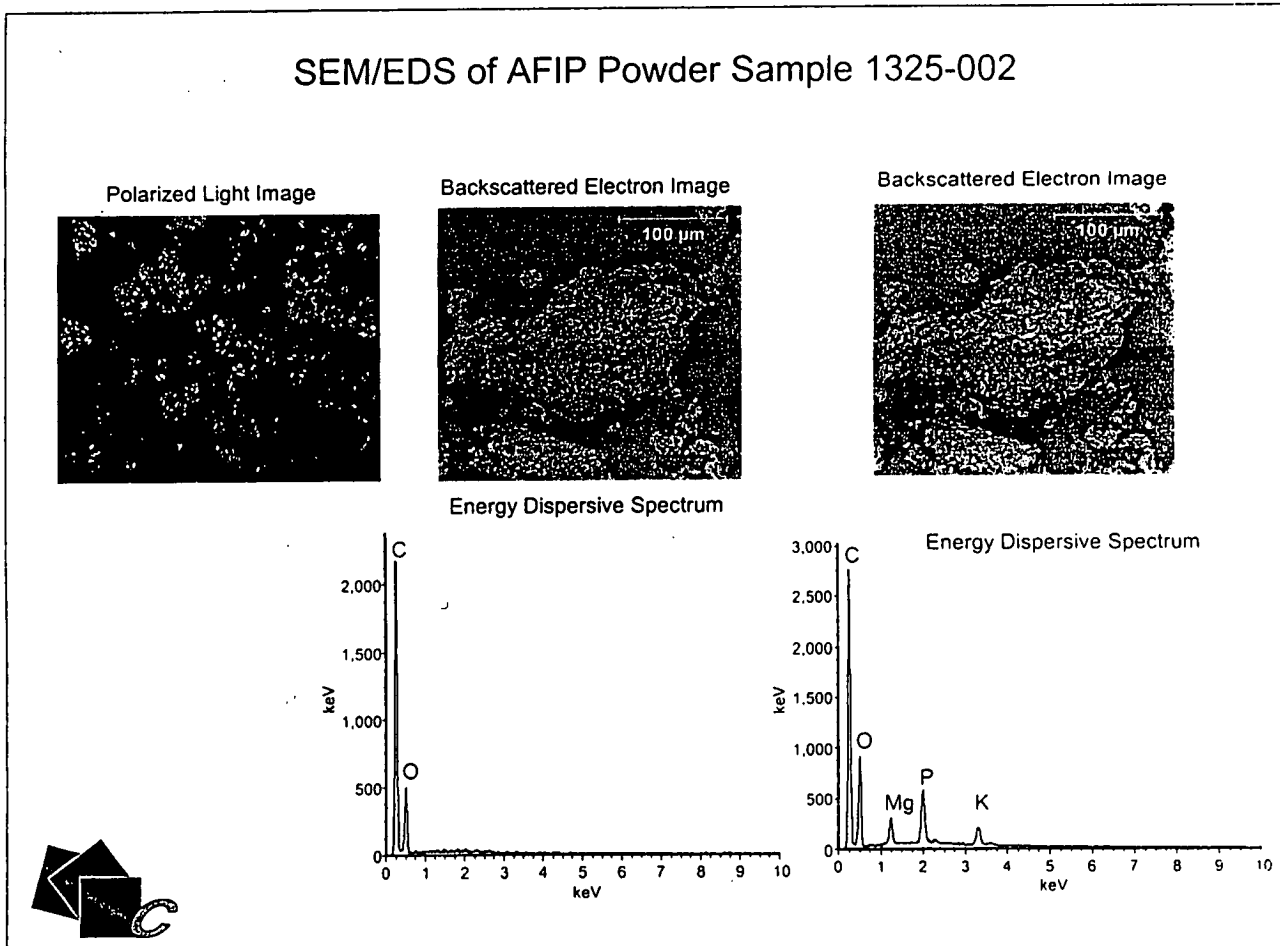

SEM/EDS of AFIP Powder Sample 1325-002
Polarized Light Image
Backscattered Electron Image
100 µm
Backscattered Electron Image
100 µm
Energy Dispersive Spectrum
C
O
keV
Energy Dispersive Spectrum
C
O
Mg
P
K
keV

Figure 5F

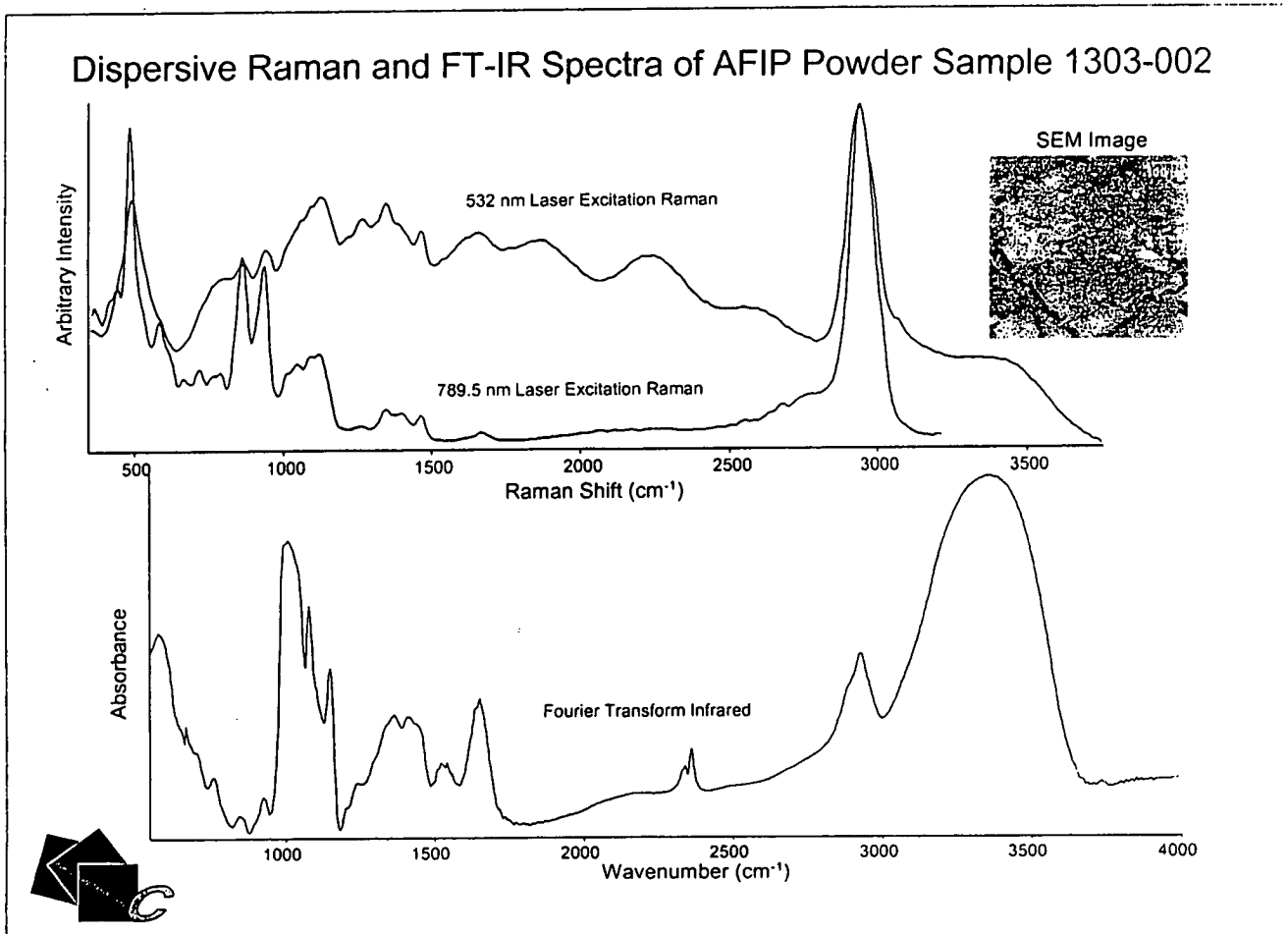

Dispersive Raman and FT-IR Spectra of AFIP Powder Sample 1303-002
SEM Image
Arbitrary Intensity
532 nm Laser Excitation Raman
789.5 nm Laser Excitation Raman
500
1000
1500
2000
2500
3000
3500
Raman Shift (cm-1)
Absorbance
Fourier Transform Infrared
1000
1500
2000
2500
3000
3500
4000
Wavenumber (cm-1)

Figure 5G

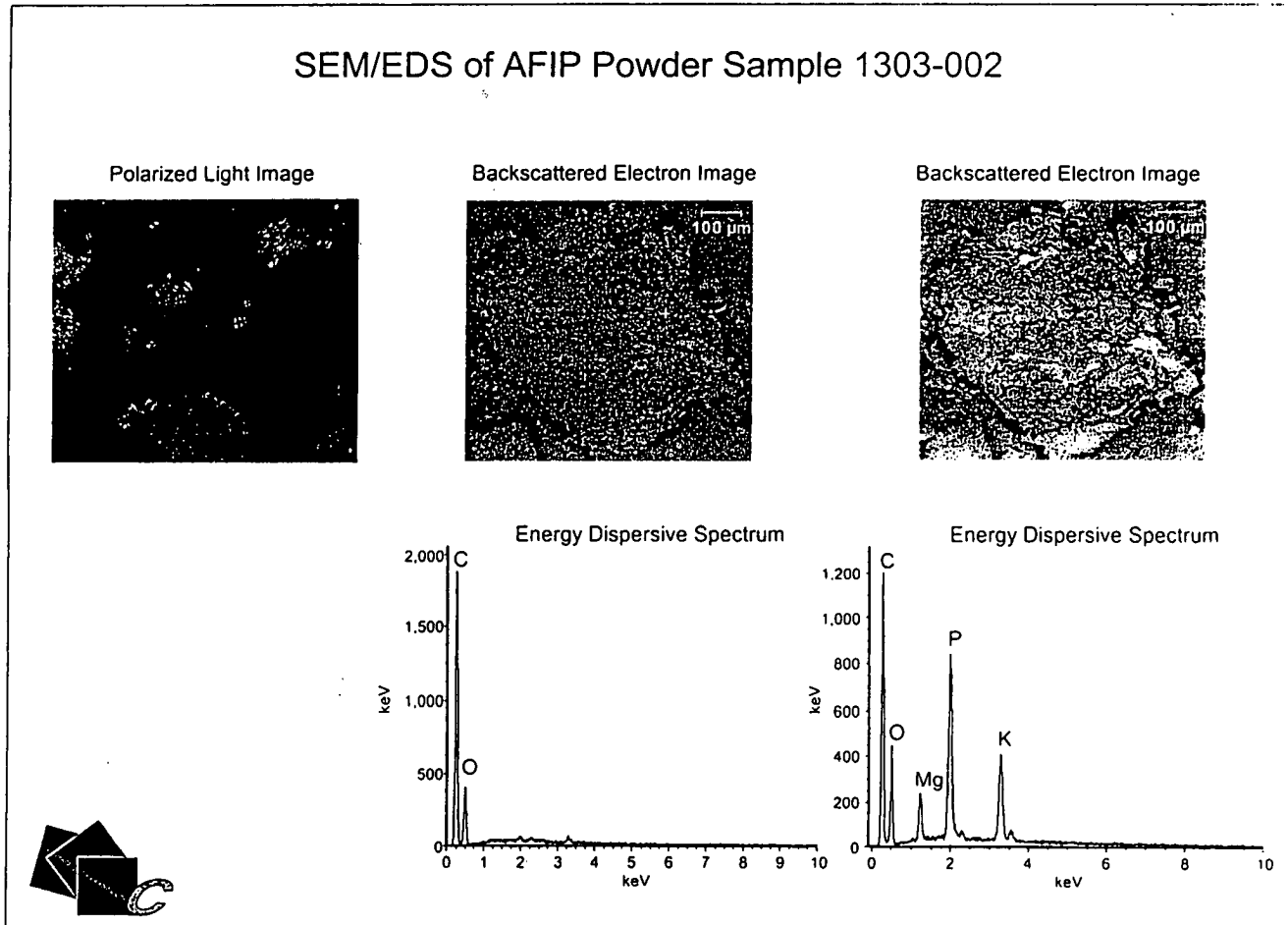

SEM/EDS of AFIP Powder Sample 1303-002
Polarized Light Image
Backscattered Electron Image
100 µm
Backscattered Electron Image
100 µm
Energy Dispersive Spectrum
2,000
1,500
1,000
500
0
keV
C
O
0 1 2 3 4 5 6 7 8 9 10
keV
Energy Dispersive Spectrum
1,200
1,000
800
600
400
200
0
keV
C
O
Mg
P
K
0 2 4 6 8 10
keV

Figure 5H

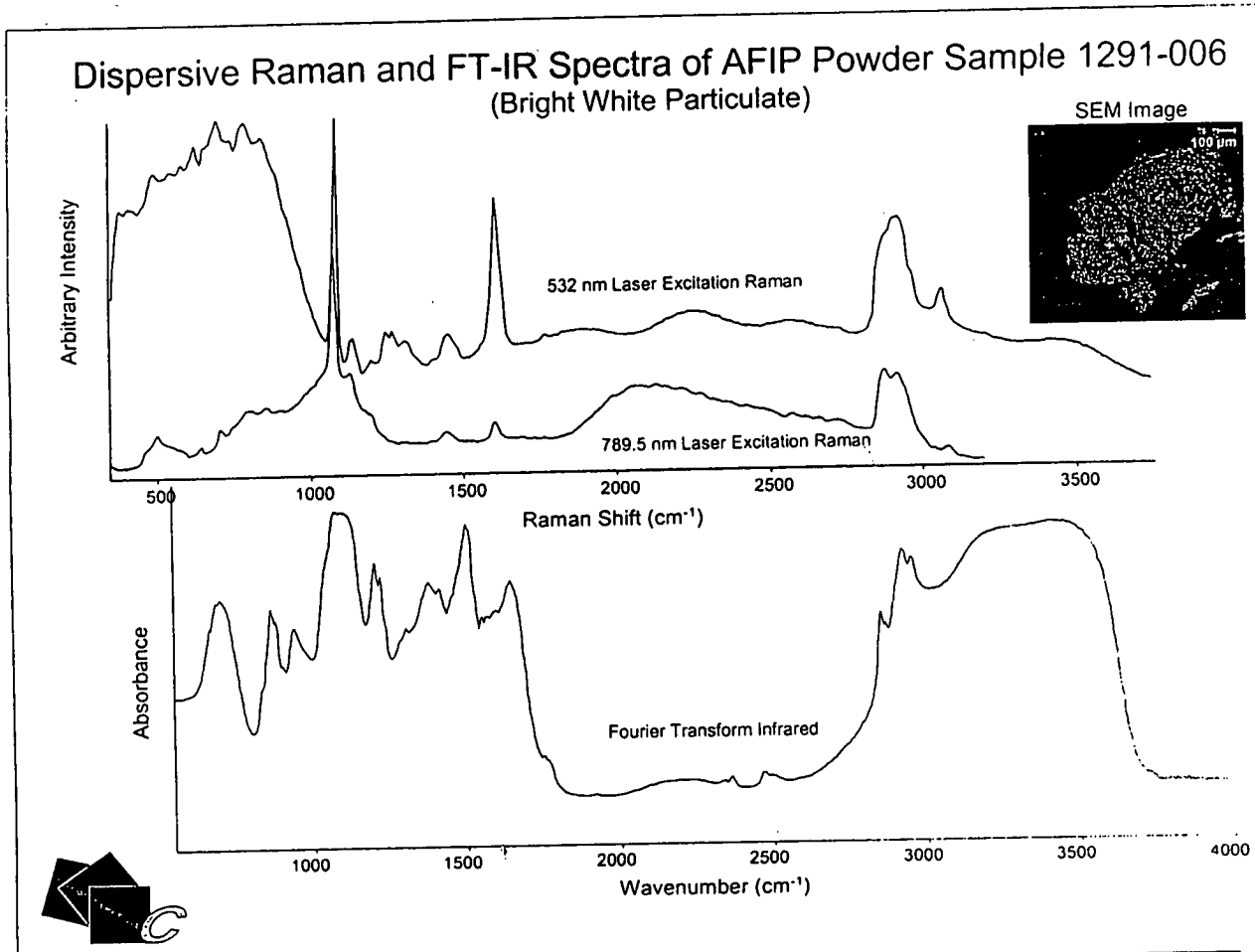
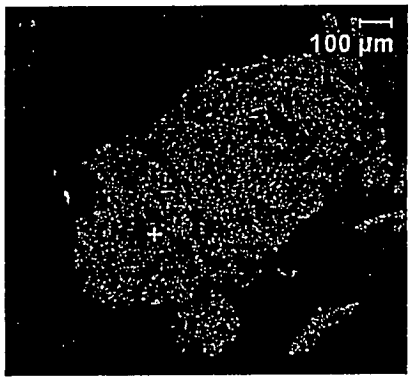

Dispersive Raman and FT-IR Spectra of AFIP Powder Sample 1291-006
(Bright White Particulate)
SEM Image
100 µm
Arbitrary Intensity
532 nm Laser Excitation Raman
789.5 nm Laser Excitation Raman
500
1000
1500
2000
2500
3000
3500
Raman Shift (cm-1)
Absorbance
Fourier Transform Infrared
1000
1500
2000
2500
3000
3500
4000
Wavenumber (cm-1)

Figure 5I

SEM/EDS of AFIP Powder Sample 1291-006
(Bright White Particulate)

Backscattered Electron Image



Energy Dispersive Spectrum

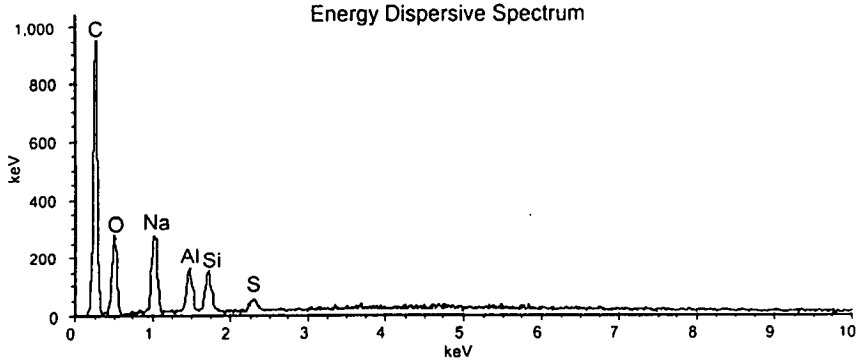


Figure 5J

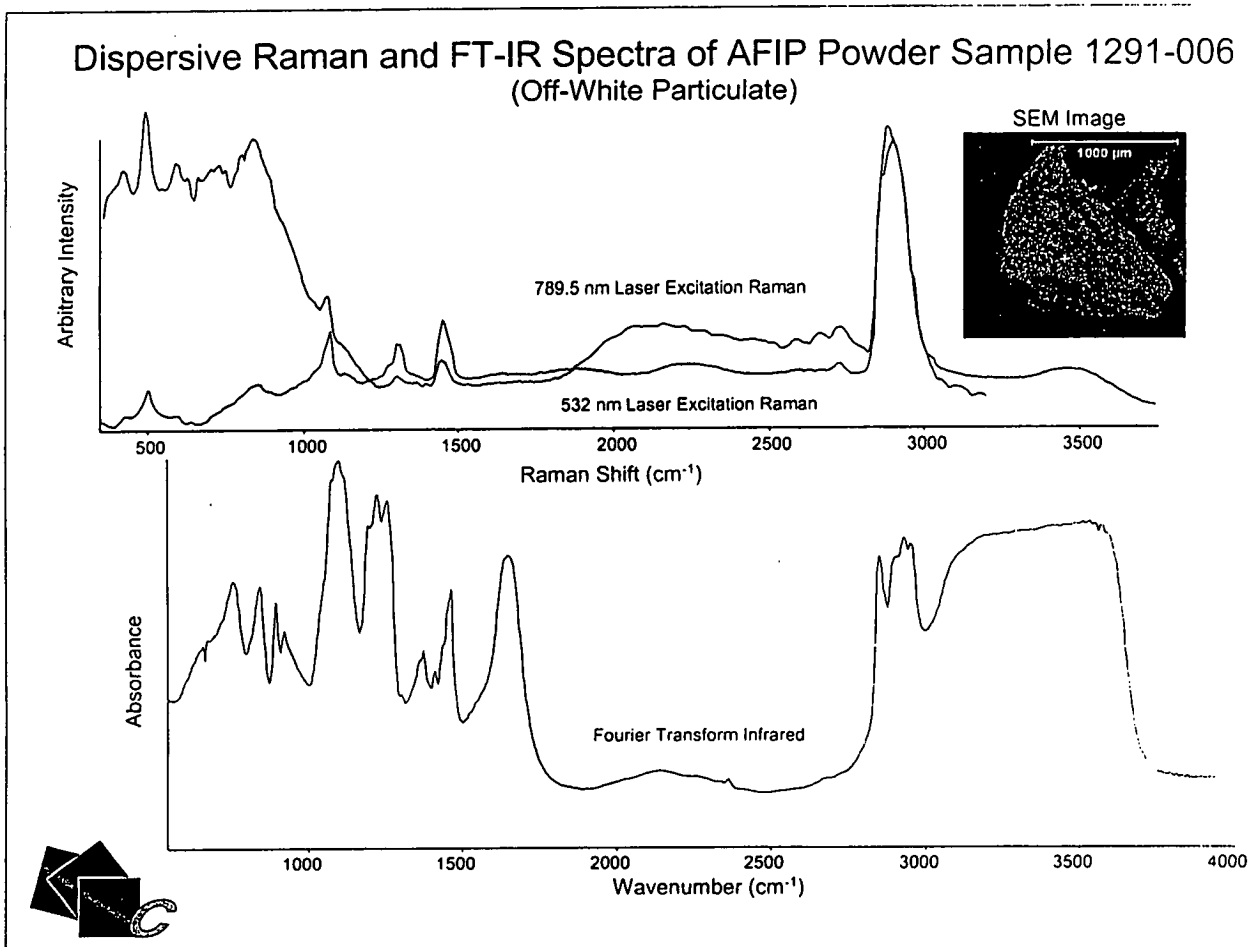
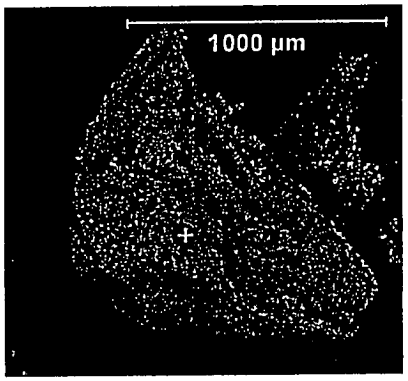

Dispersive Raman and FT-IR Spectra of AFIP Powder Sample 1291-006
(Off-White Particulate)
SEM Image
1000 µm
Arbitrary Intensity
789.5 nm Laser Excitation Raman
532 nm Laser Excitation Raman
500
1000
1500
2000
2500
3000
3500
Raman Shift (cm-1)
Absorbance
Fourier Transform Infrared
1000
1500
2000
2500
3000
3500
4000
Wavenumber (cm-1)

Figure 5K

SEM/EDS of AFIP Powder Sample 1291-006

(Off-White Particulate)

Backscattered Electron Image



Energy Dispersive Spectrum

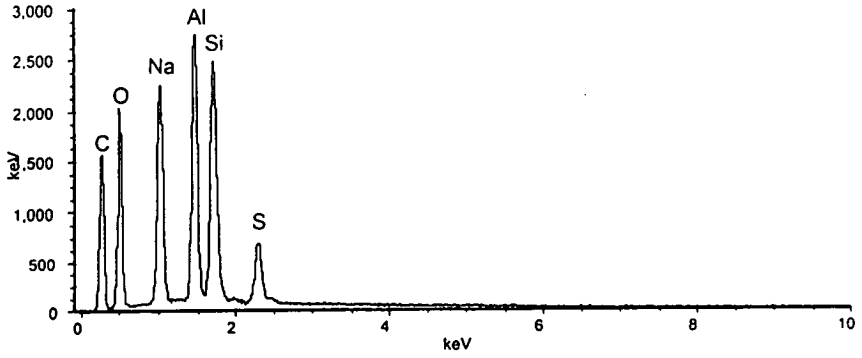


Figure 5L

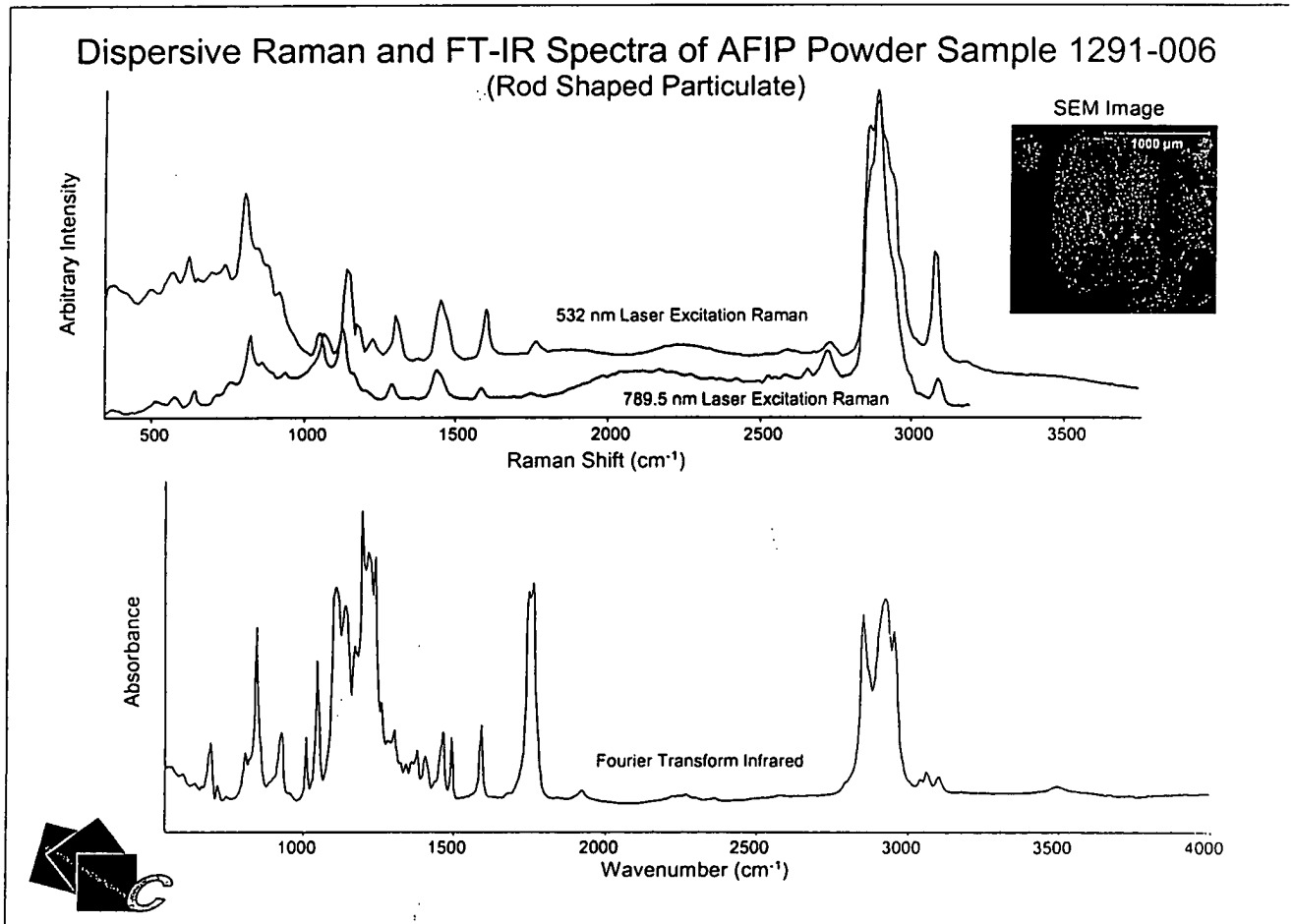
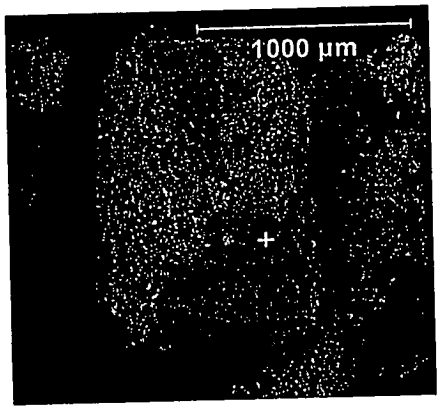

Dispersive Raman and FT-IR Spectra of AFIP Powder Sample 1291-006
(Rod Shaped Particulate)
SEM Image
1000 µm
Arbitrary Intensity
532 nm Laser Excitation Raman
789.5 nm Laser Excitation Raman
500
1000
1500
2000
2500
3000
3500
Raman Shift (cm-1)
Absorbance
Fourier Transform Infrared
1000
1500
2000
2500
3000
3500
4000
Wavenumber (cm-1)

Figure 5M

SEM/EDS of AFIP Powder Sample 1291-006
(Rod-Shaped Particulate)

Backscattered Electron Image



Energy Dispersive Spectrum

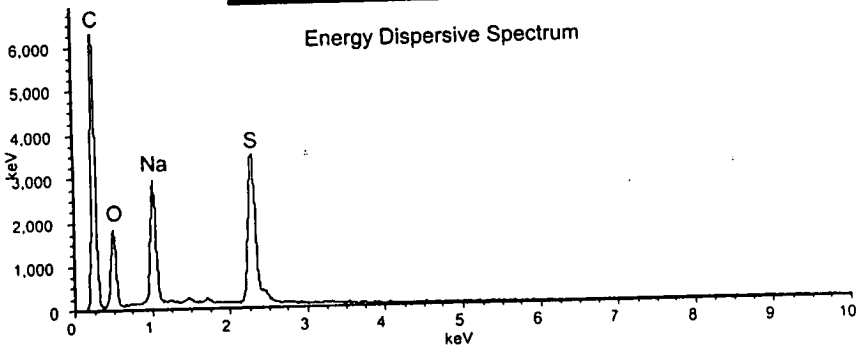


Figure 5N

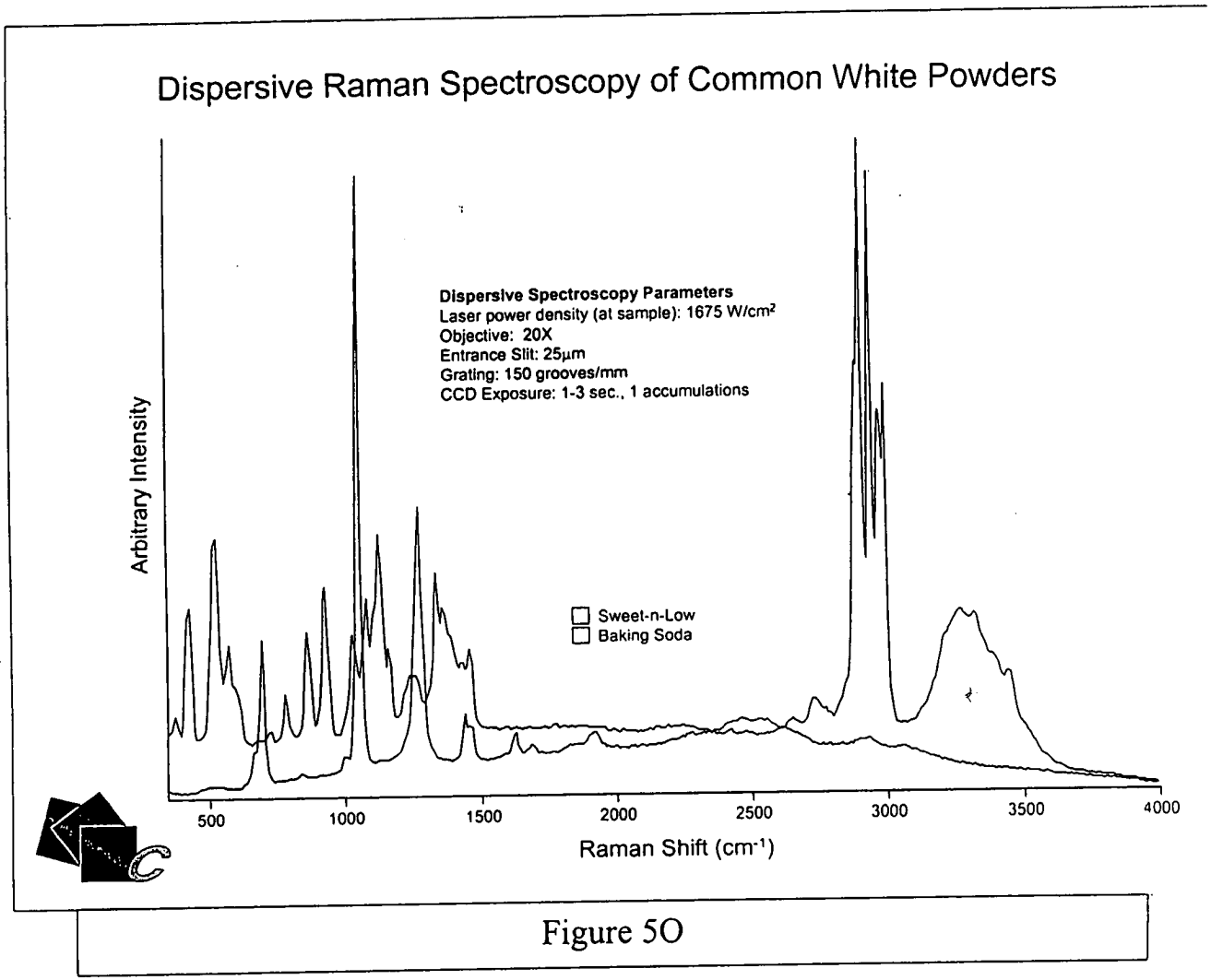

Dispersive Raman Spectroscopy of Common White Powders
Dispersive Spectroscopy Parameters
Laser power density (at sample): 1675 W/cm²
Objective: 20X
Entrance Slit: 25µm
Grating: 150 grooves/mm
CCD Exposure: 1-3 sec., 1 accumulations
Arbitrary Intensity
Sweet-n-Low
Baking Soda
500
1000
1500
2000
2500
3000
3500
4000
Raman Shift (cm⁻¹)

Figure 5O

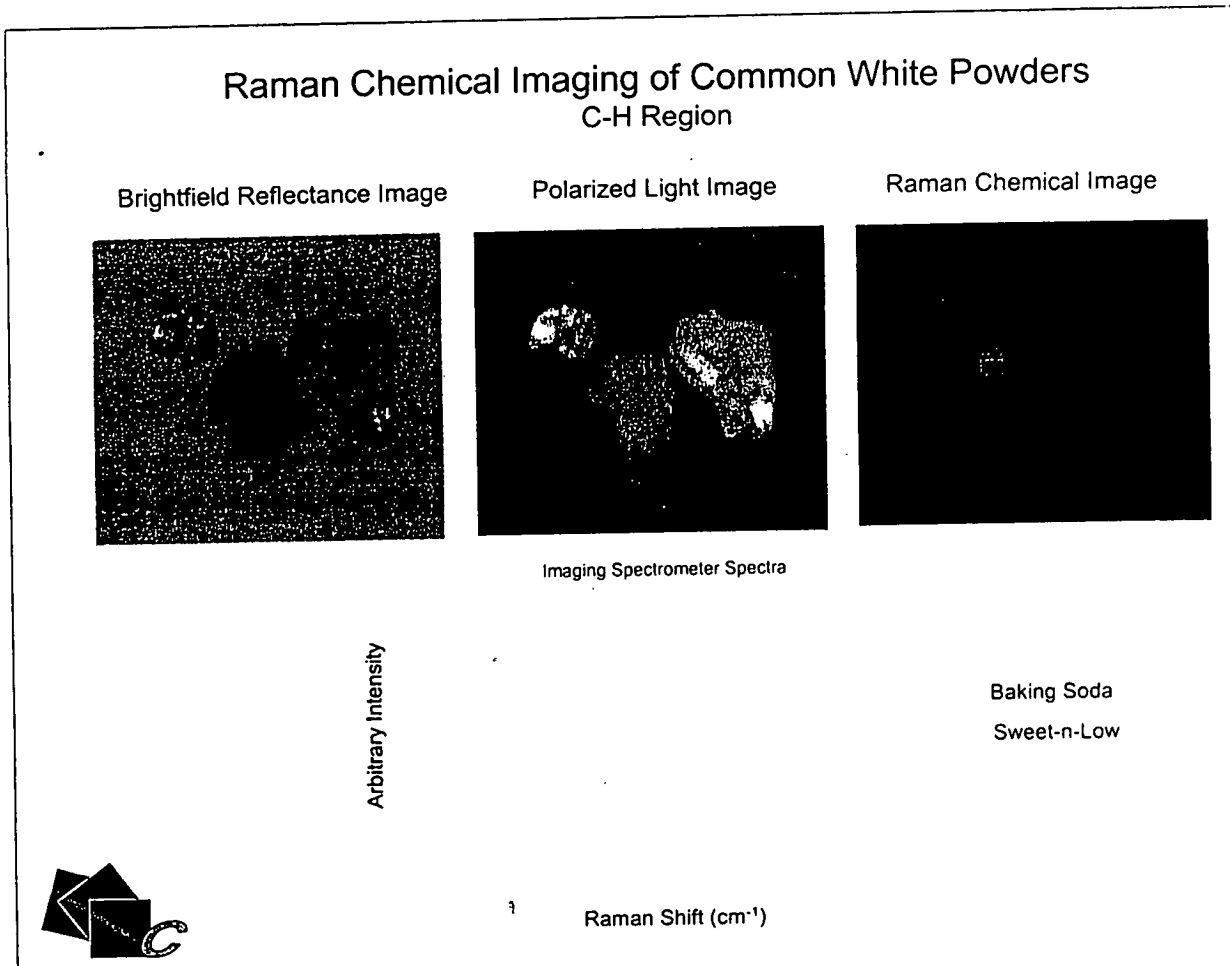

Raman Chemical Imaging of Common White Powders
C-H Region
Brightfield Reflectance Image
Polarized Light Image
Raman Chemical Image
Imaging Spectrometer Spectra
Arbitrary Intensity
Baking Soda
Sweet-n-Low
Raman Shift (cm-1)

Figure 5P

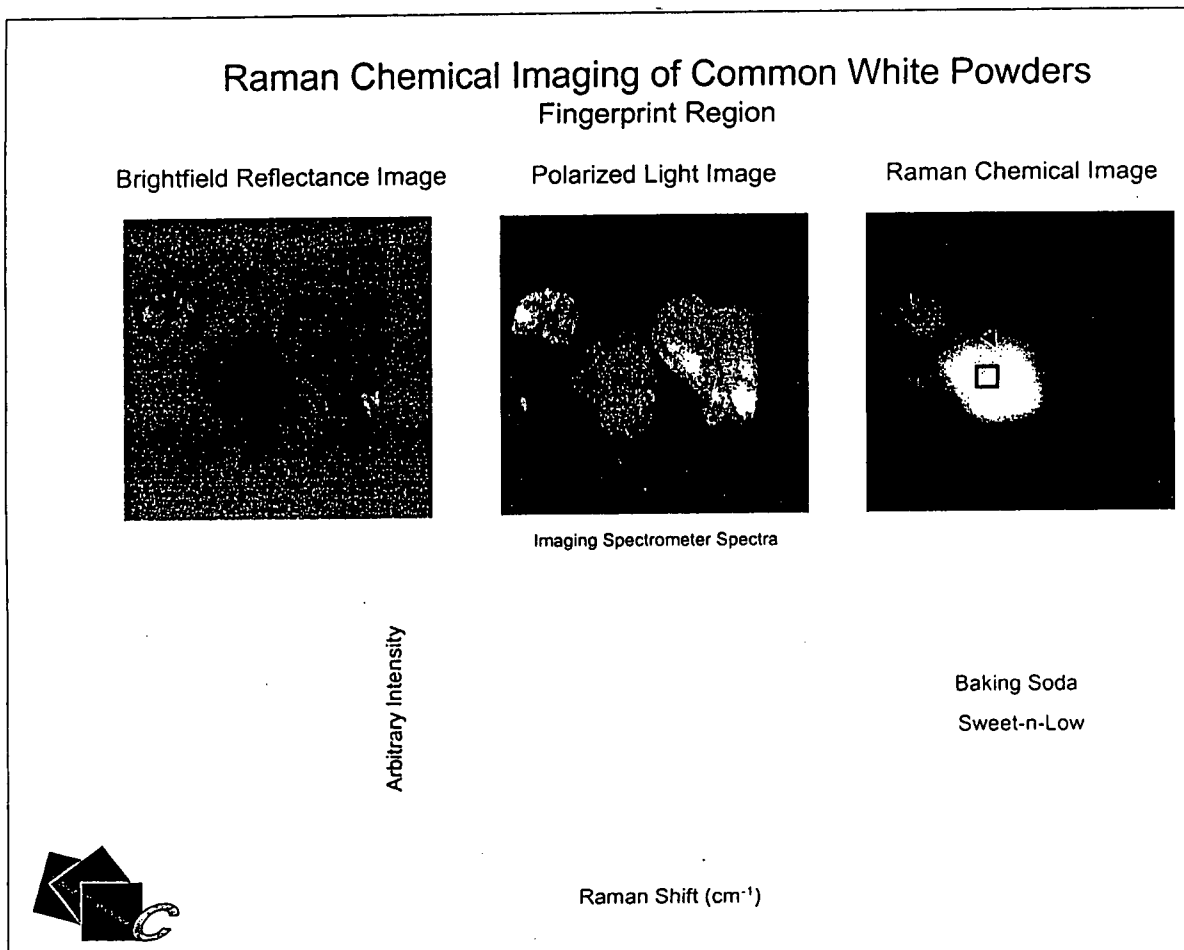

Raman Chemical Imaging of Common White Powders
Fingerprint Region
Brightfield Reflectance Image
Polarized Light Image
Raman Chemical Image
Imaging Spectrometer Spectra
Arbitrary Intensity
Baking Soda
Sweet-n-Low
Raman Shift (cm-1)

Figure 5Q

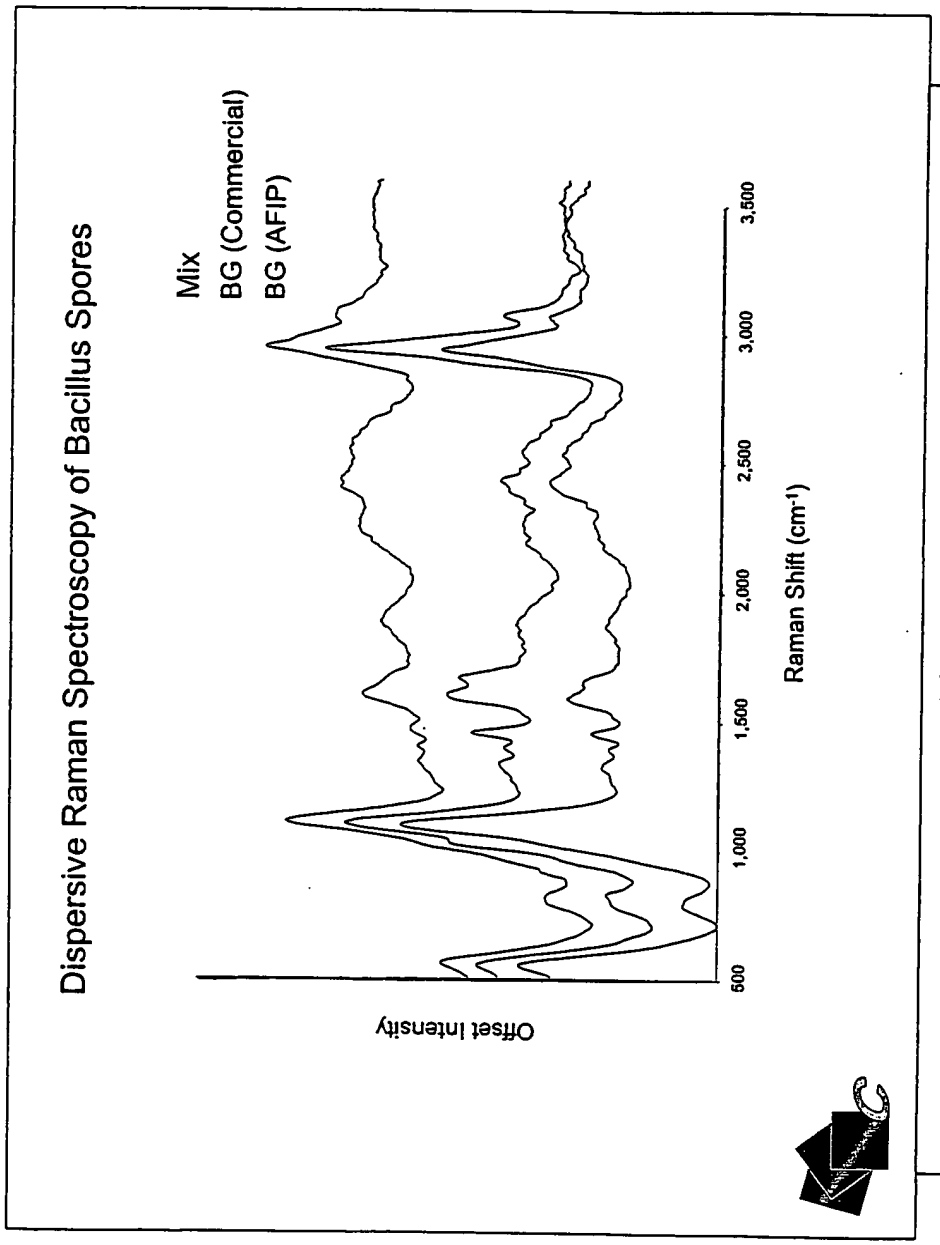

Dispersive Raman Spectroscopy of Bacillus Spores
Mix
BG (Commercial)
BG (AFIP)
Offset Intensity
500
1,000
1,500
2,000
2,500
3,000
3,500
Raman Shift (cm-1)

Figure 5R

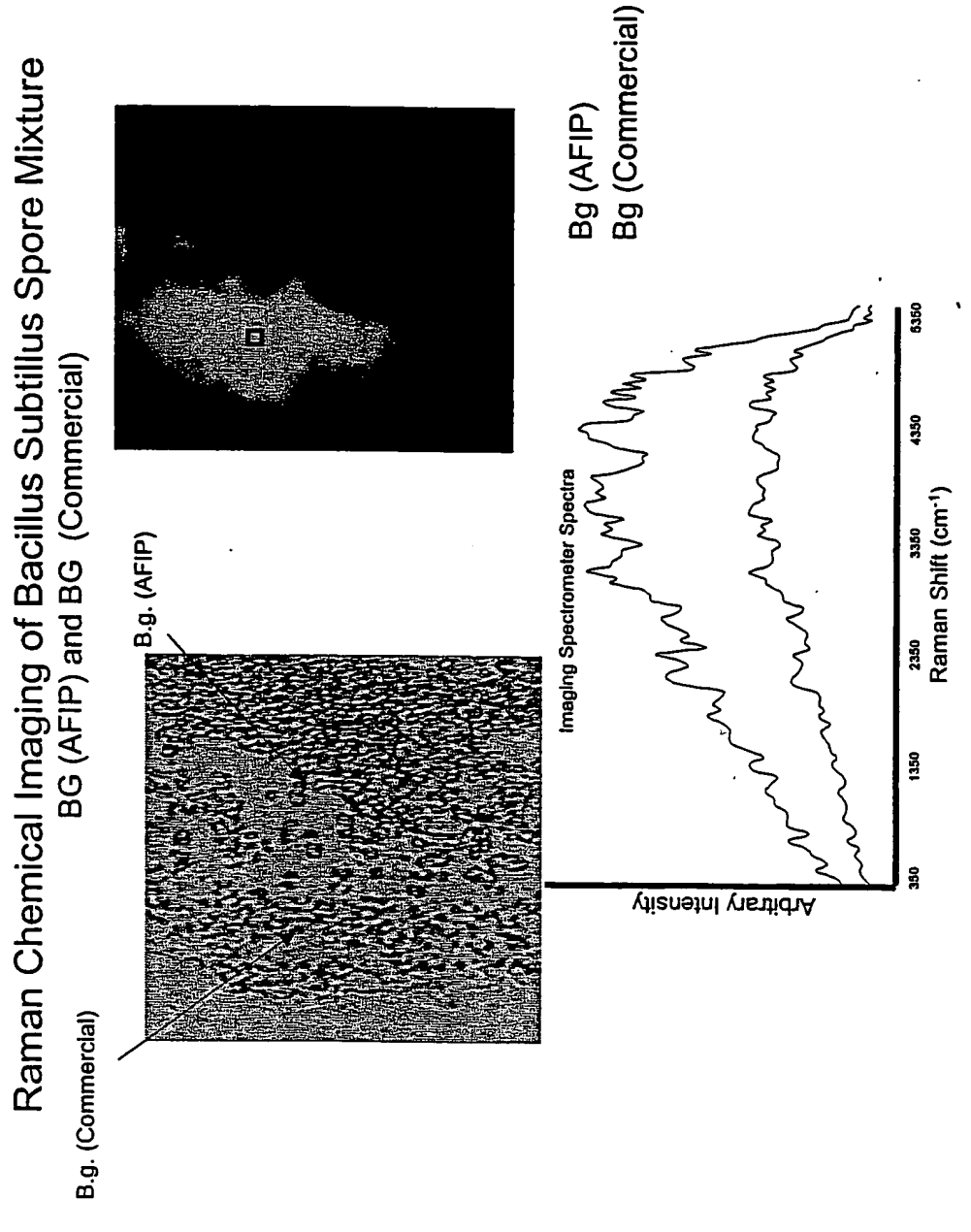

Raman Chemical Imaging of Bacillus Subtillus Spore Mixture
BG (AFIP) and BG (Commercial)
B.g. (Commercial)
B.g. (AFIP)
Imaging Spectrometer Spectra
Bg (AFIP)
Bg (Commercial)
Arbitrary Intensity
350
1350
2350
3350
4350
5350
Raman Shift (cm-1)

Figure 5S

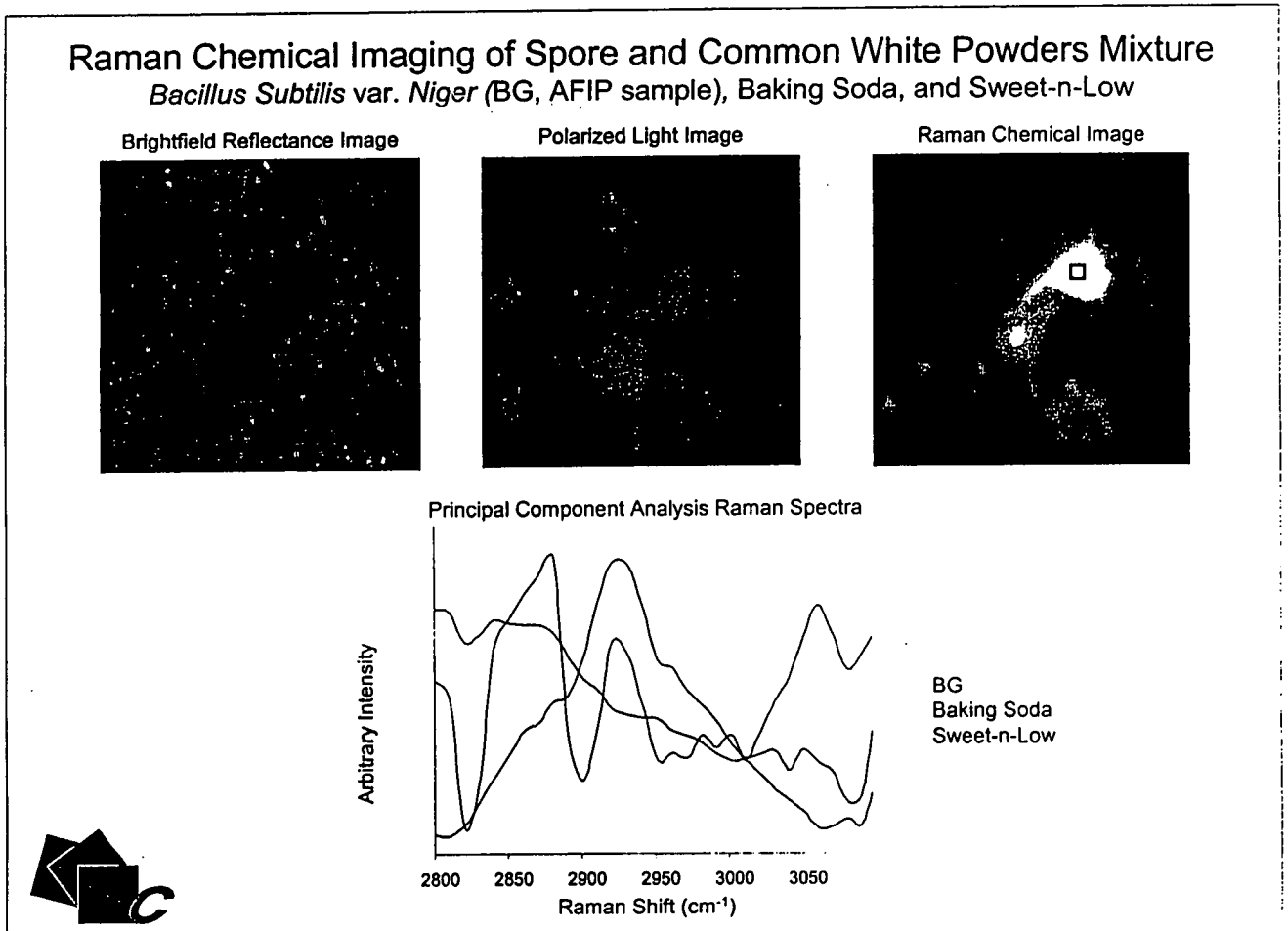

Raman Chemical Imaging of Spore and Common White Powders Mixture
Bacillus Subtilis var. Niger (BG, AFIP sample), Baking Soda, and Sweet-n-Low
Brightfield Reflectance Image
Polarized Light Image
Raman Chemical Image
Principal Component Analysis Raman Spectra
Arbitrary Intensity
2800
2850
2900
2950
3000
3050
Raman Shift (cm-1)
BG
Baking Soda
Sweet-n-Low

Figure 5T

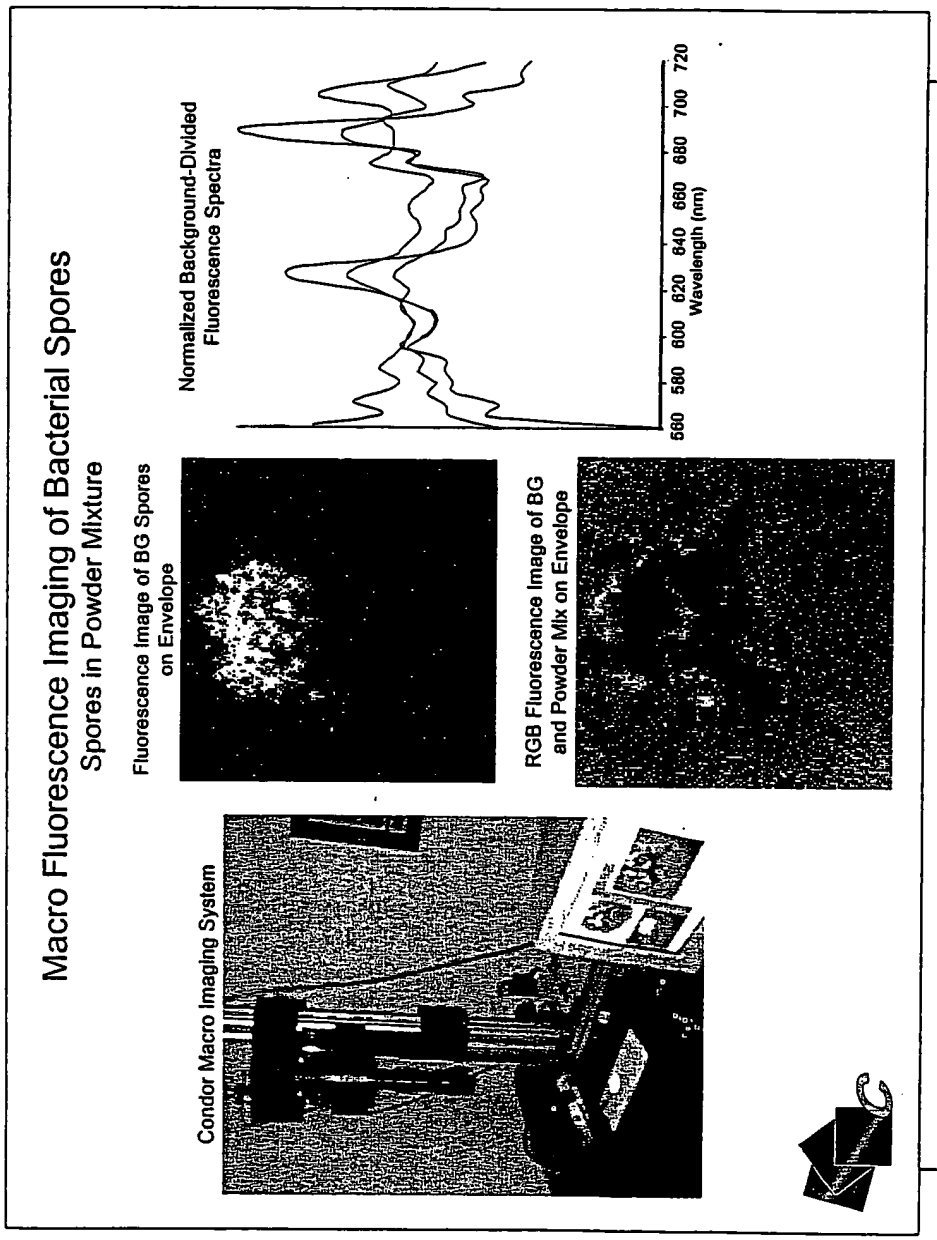

Macro Fluorescence Imaging of Bacterial Spores
Spores in Powder Mixture
Condor Macro Imaging System
Fluorescence Image of BG Spores on Envelope
RGB Fluorescence Image of BG and Powder Mix on Envelope
Normalized Background-Divided Fluorescence Spectra
560 580 600 620 640 660 680 700 720
Wavelength (nm)

Figure 5U

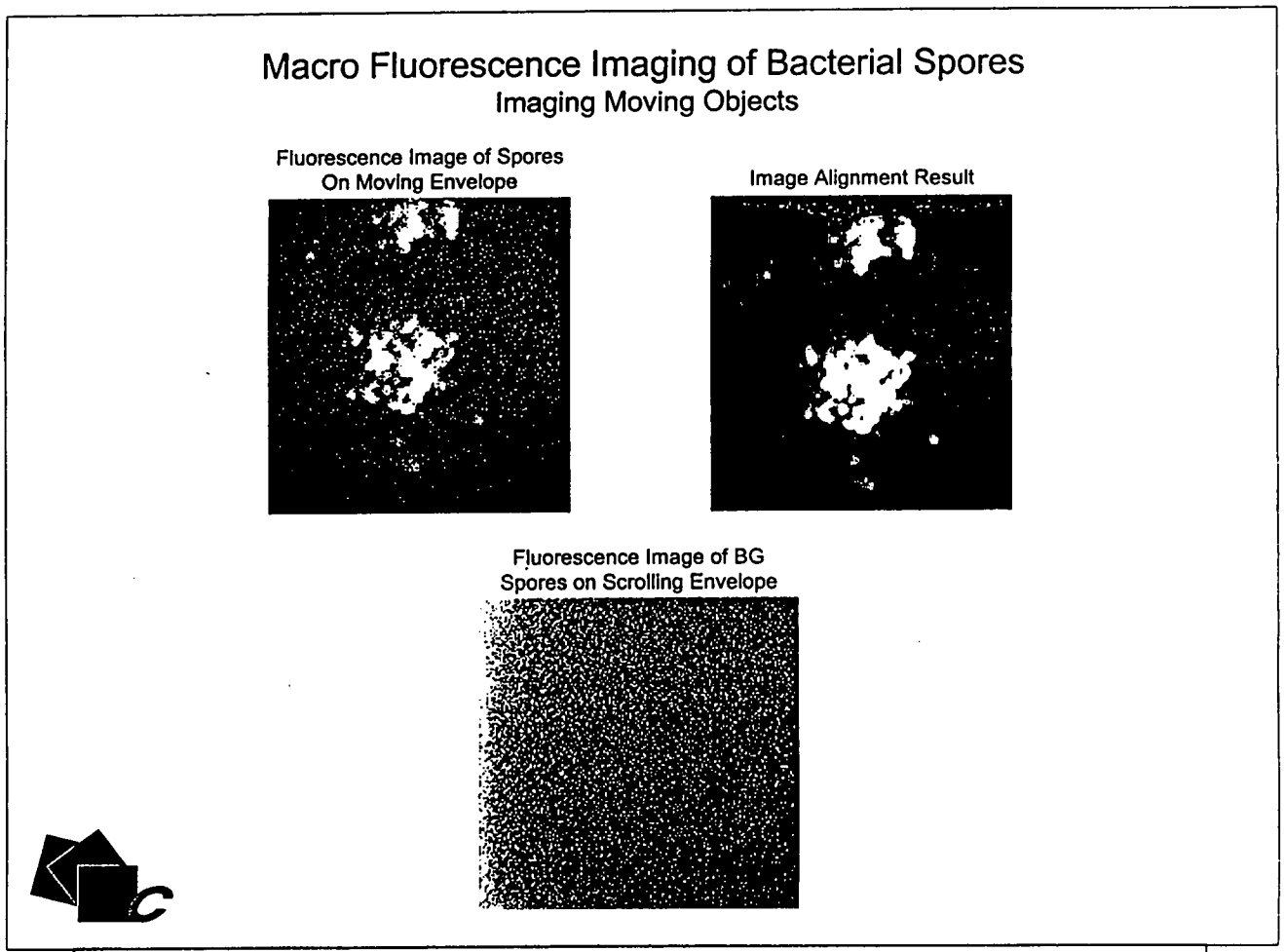

Macro Fluorescence Imaging of Bacterial Spores
Imaging Moving Objects
Fluorescence Image of Spores
On Moving Envelope
Image Alignment Result
Fluorescence Image of BG
Spores on Scrolling Envelope

Figure 5V

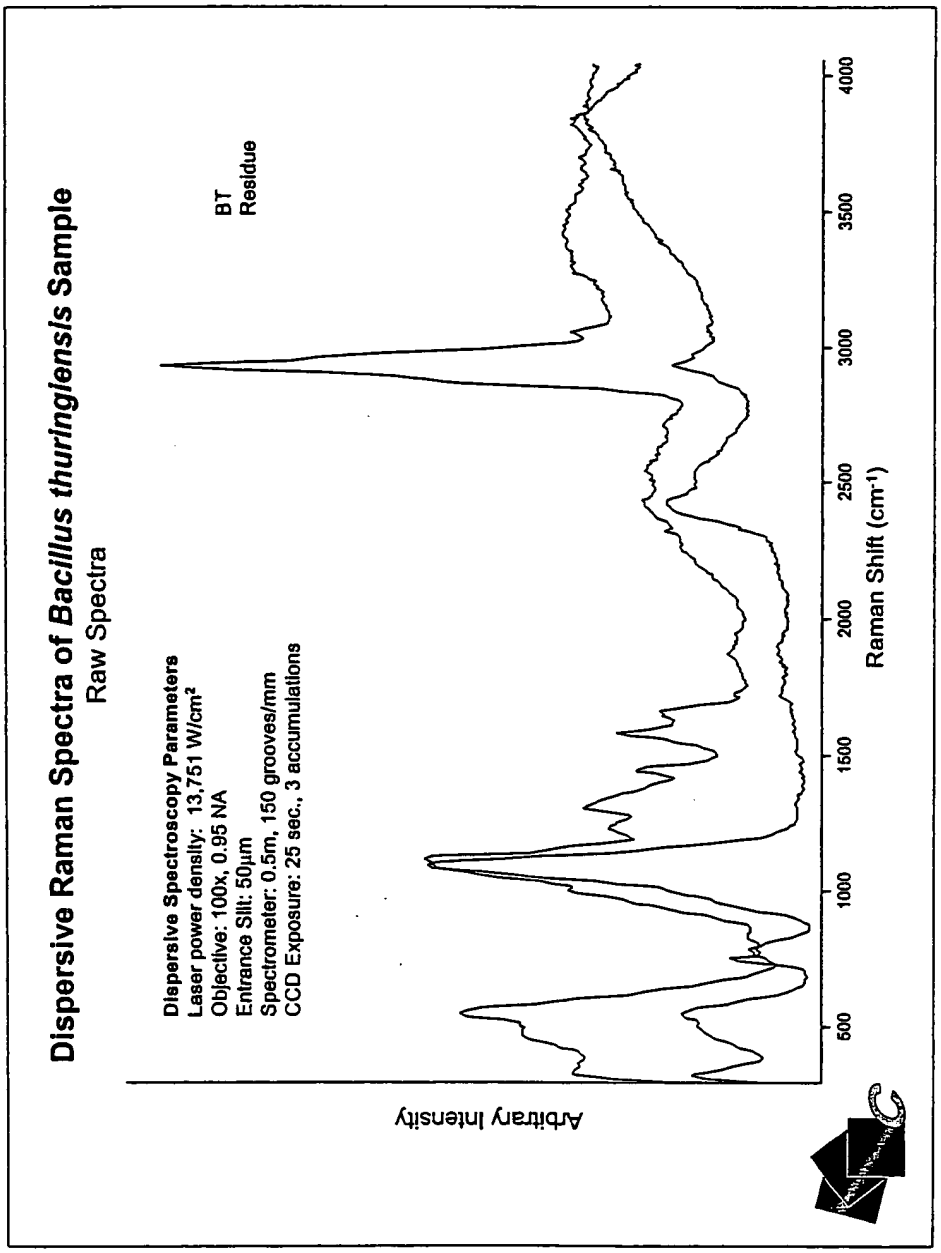

Dispersive Raman Spectra of Bacillus thuringiensis Sample
Raw Spectra
Dispersive Spectroscopy Parameters
Laser power density: 13,751 W/cm²
Objective: 100x, 0.95 NA
Entrance Slit: 50µm
Spectrometer: 0.5m, 150 grooves/mm
CCD Exposure: 25 sec., 3 accumulations
BT
Residue
Arbitrary Intensity
500
1000
1500
2000
2500
3000
3500
4000
Raman Shift (cm-1)

Figure 6A

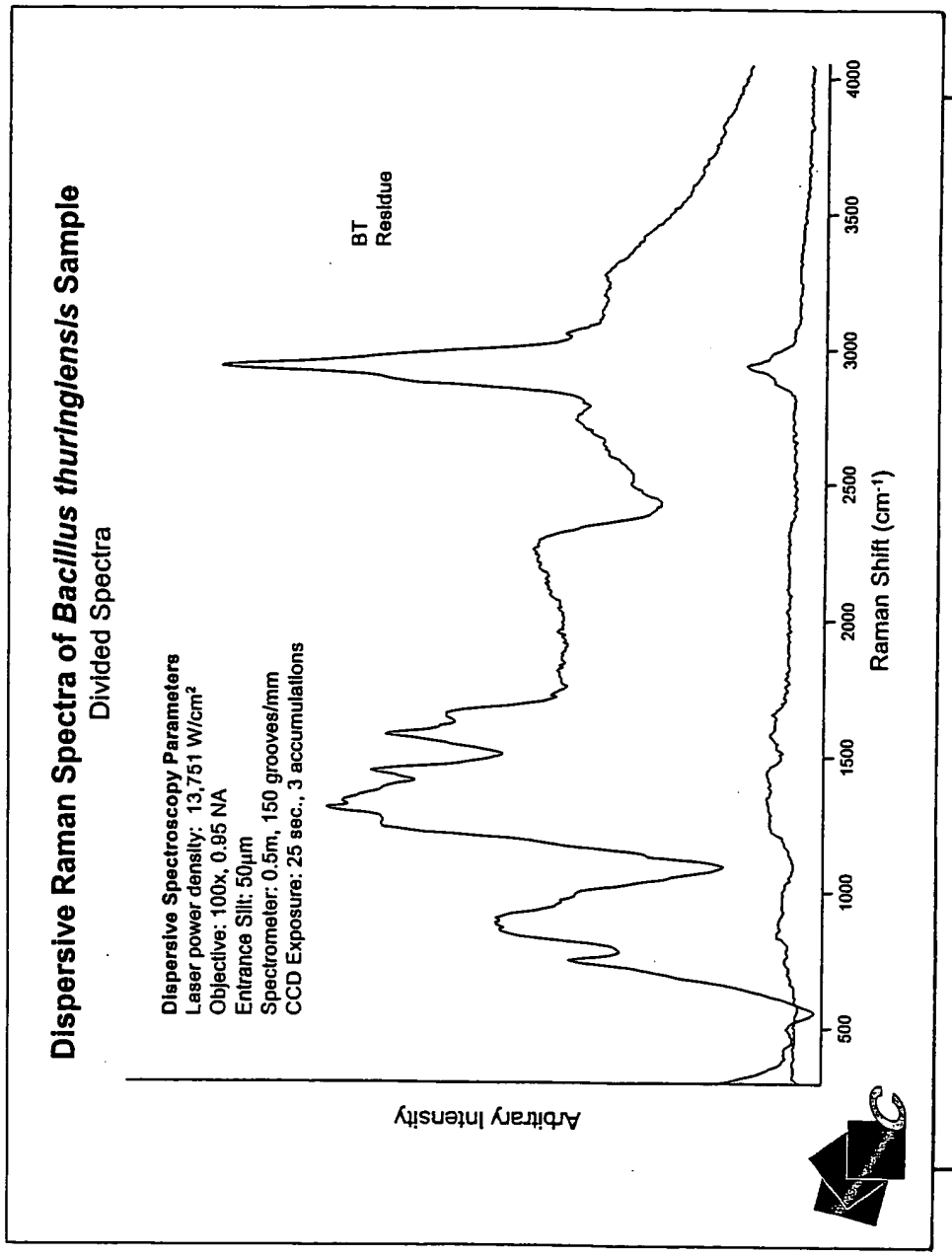

Dispersive Raman Spectra of Bacillus thuringlensis Sample
Divided Spectra
Dispersive Spectroscopy Parameters
Laser power density: 13,751 W/cm²
Objective: 100x, 0.95 NA
Entrance Slit: 50µm
Spectrometer: 0.5m, 150 grooves/mm
CCD Exposure: 25 sec., 3 accumulations
BT
Residue
Arbitrary Intensity
500
1000
1500
2000
2500
3000
3500
4000
Raman Shift (cm-1)

Figure 6B

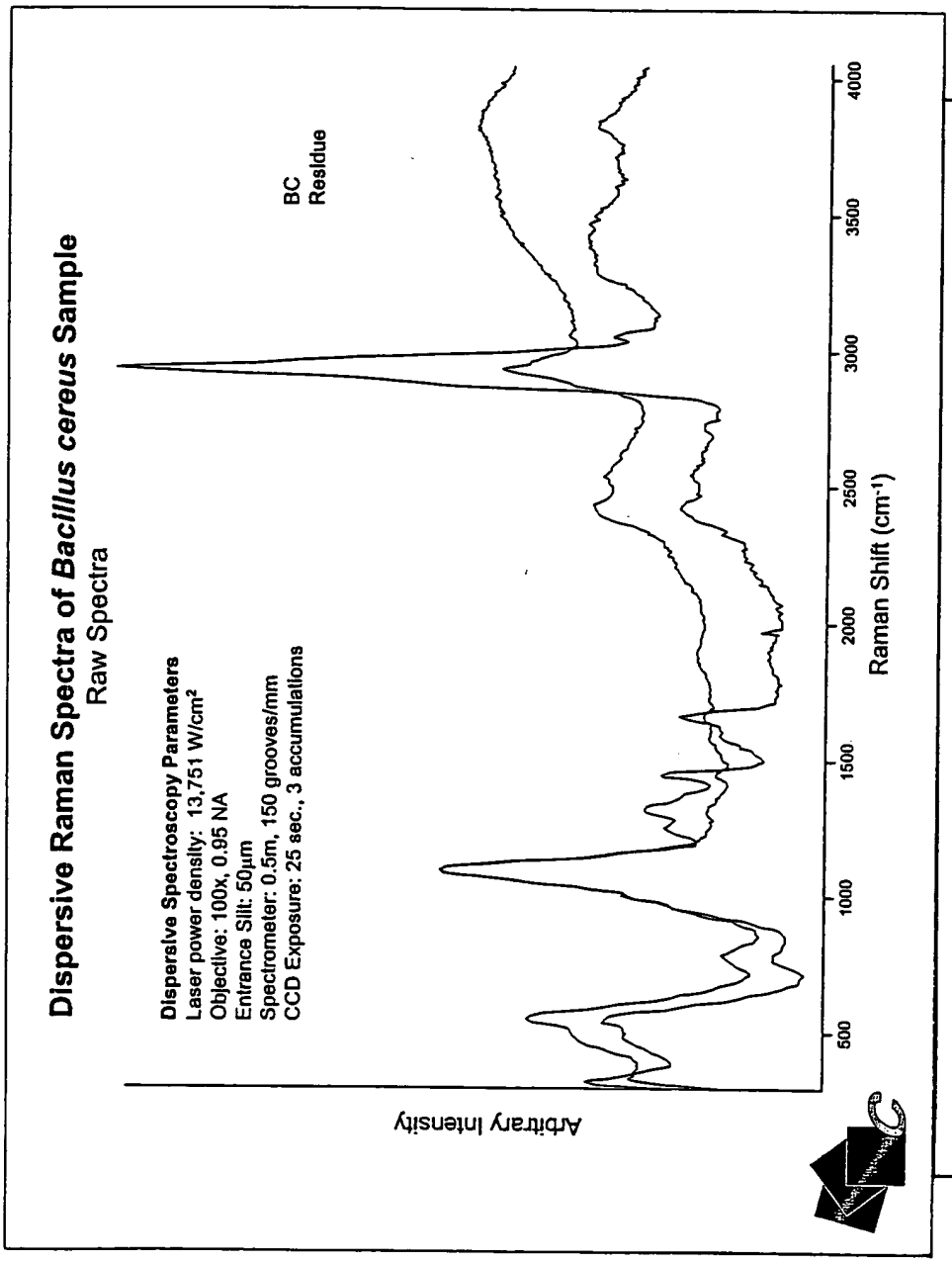

Dispersive Raman Spectra of Bacillus cereus Sample
Raw Spectra
Dispersive Spectroscopy Parameters
Laser power density: 13,751 W/cm²
Objective: 100x, 0.95 NA
Entrance Slit: 50µm
Spectrometer: 0.5m, 150 grooves/mm
CCD Exposure: 25 sec., 3 accumulations
BC
Residue
Arbitrary Intensity
500
1000
1500
2000
2500
3000
3500
4000
Raman Shift (cm-1)

Figure 6C

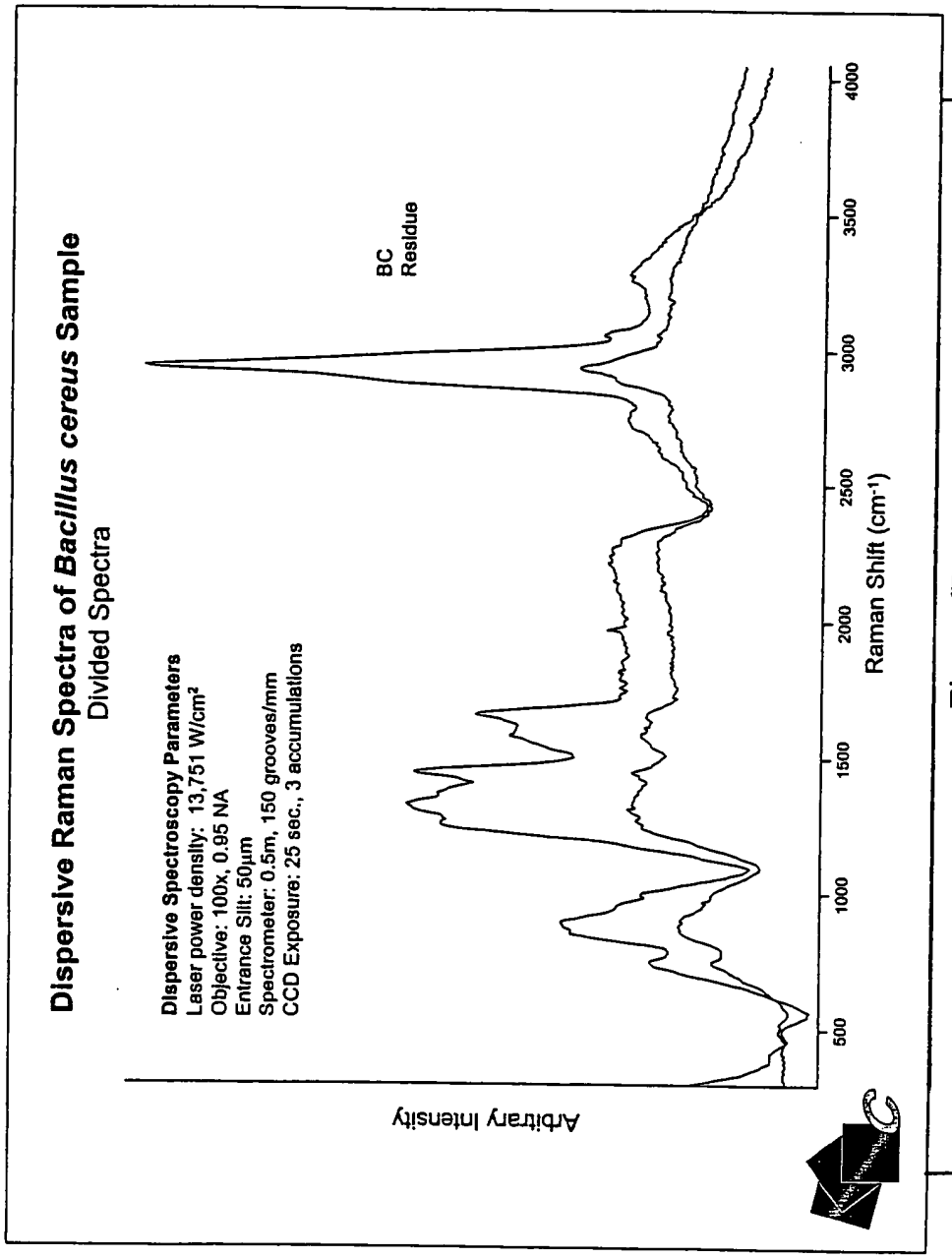

Dispersive Raman Spectra of Bacillus cereus Sample
Divided Spectra
Dispersive Spectroscopy Parameters
Laser power density: 13,751 W/cm²
Objective: 100x, 0.95 NA
Entrance Slit: 50µm
Spectrometer: 0.5m, 150 grooves/mm
CCD Exposure: 25 sec., 3 accumulations
BC
Residue
Arbitrary Intensity
500
1000
1500
2000
2500
3000
3500
4000
Raman Shift (cm-1)

Figure 6D

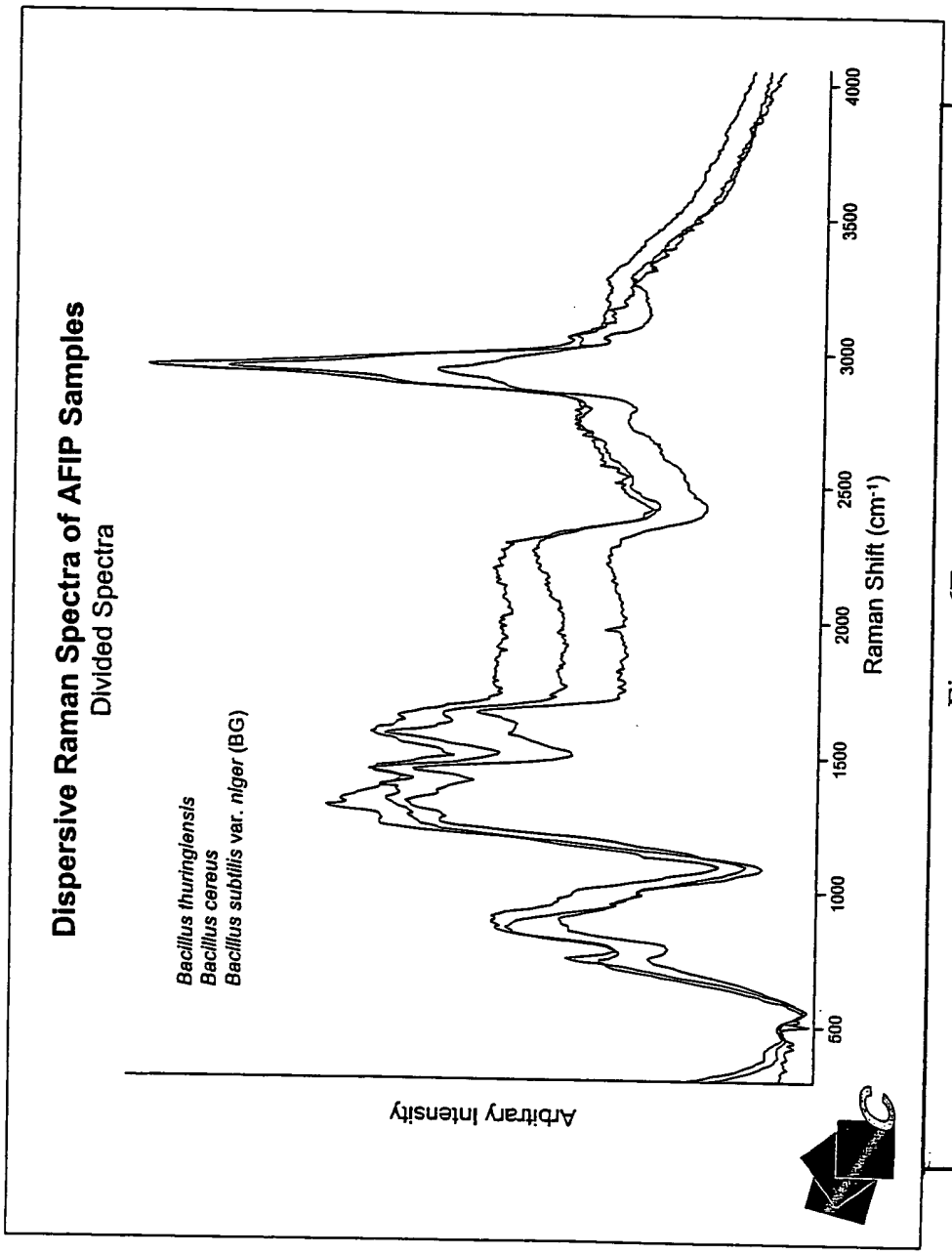

Dispersive Raman Spectra of AFIP Samples
Divided Spectra
Bacillus thuringiensis
Bacillus cereus
Bacillus subtilis var. niger (BG)
Arbitrary Intensity
600
1000
1500
2000
2500
3000
3500
4000
Raman Shift (cm-1)

Figure 6F

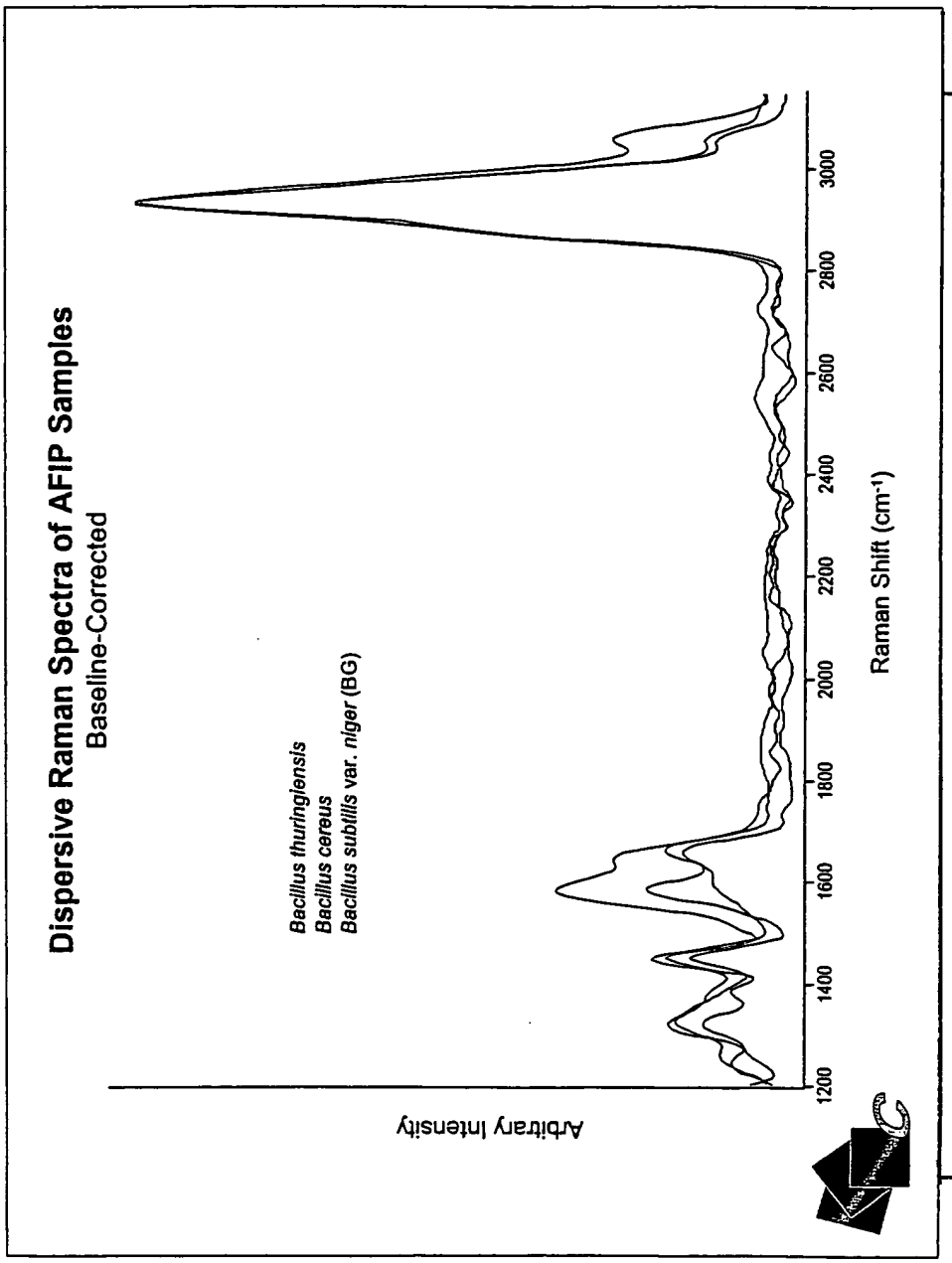

Dispersive Raman Spectra of AFIP Samples
Baseline-Corrected
Bacillus thuringiensis
Bacillus cereus
Bacillus subtilis var. niger (BG)
Arbitrary Intensity
1200
1400
1600
1800
2000
2200
2400
2600
2800
3000
Raman Shift (cm-1)

Figure 6F

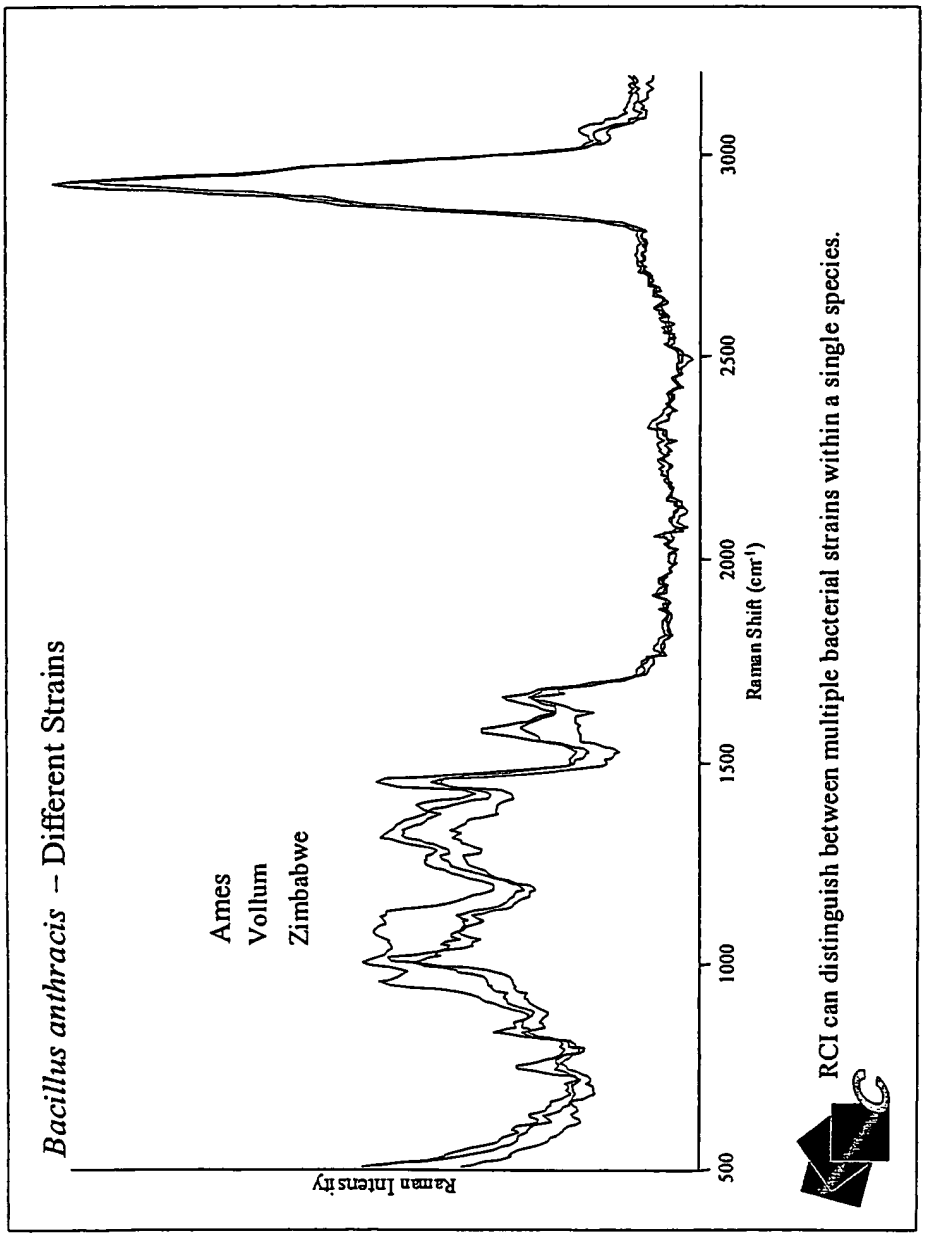

Bacillus anthracis – Different Strains
Ames
Vollum
Zimbabwe
Raman Intensity
500
1000
1500
2000
2500
3000
Raman Shift (cm-1)
RCI can distinguish between multiple bacterial strains within a single species.

Figure 7

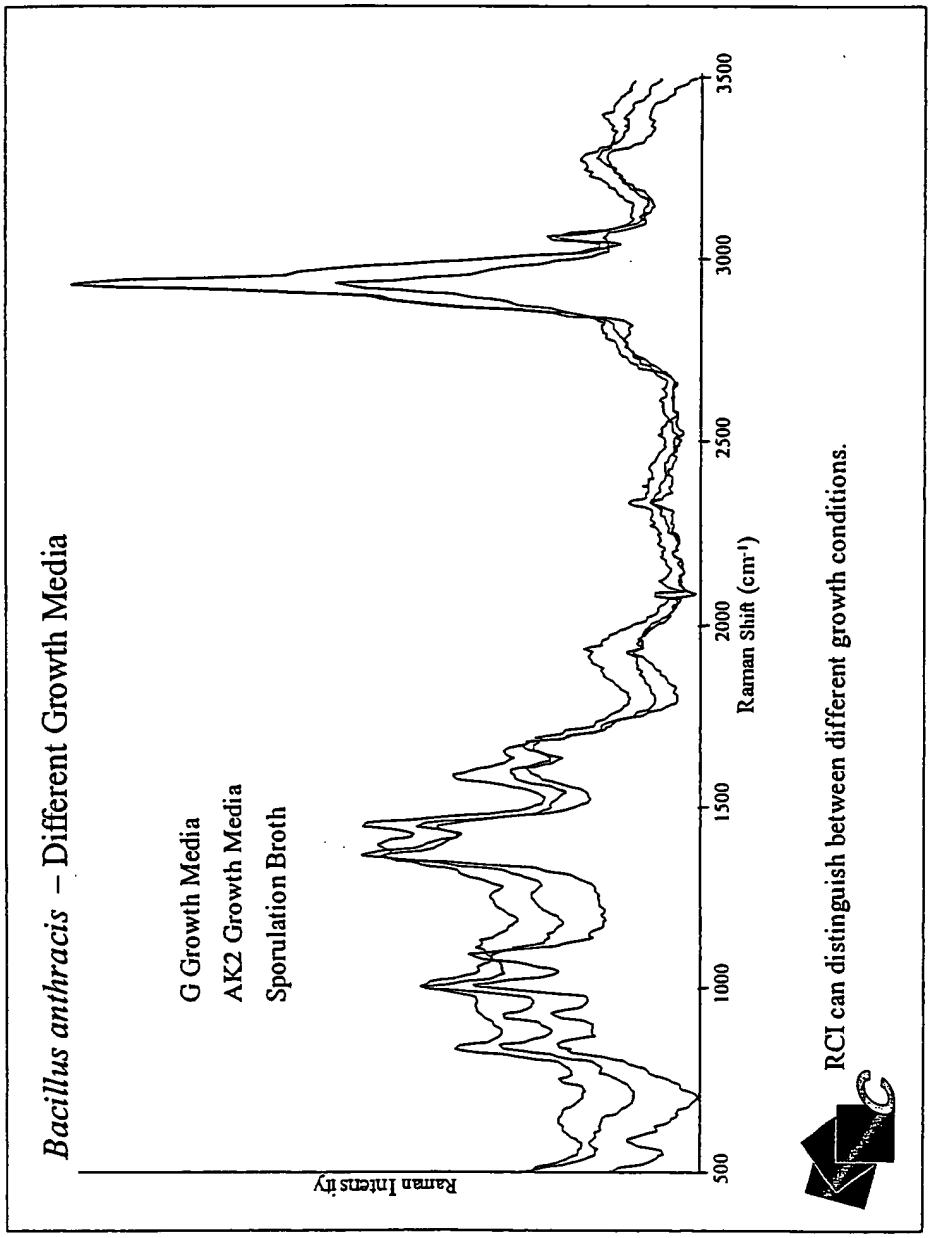
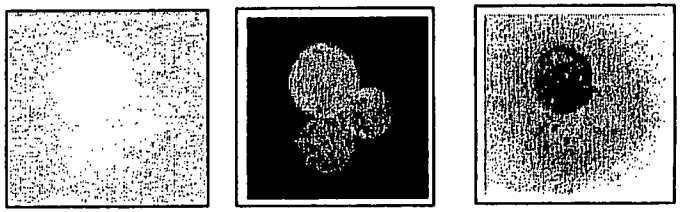
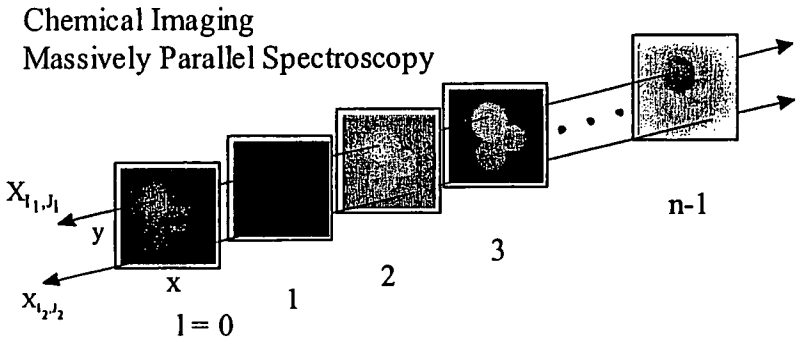

Bacillus anthracis – Different Growth Media
G Growth Media
AK2 Growth Media
Sporulation Broth
Raman Intensity
500
1000
1500
2000
2500
3000
3500
Raman Shift (cm-1)
RCI can distinguish between different growth conditions.

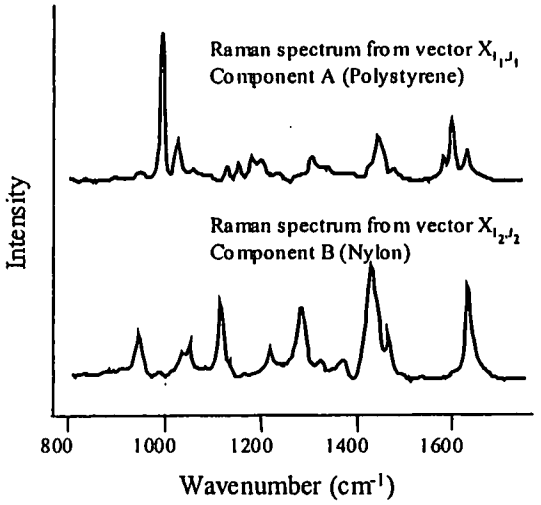
Figure 8

Why Chemical Imaging?

- Fast
- Minimally invasive
- High information content
- Spectroscopy provides fingerprint for material

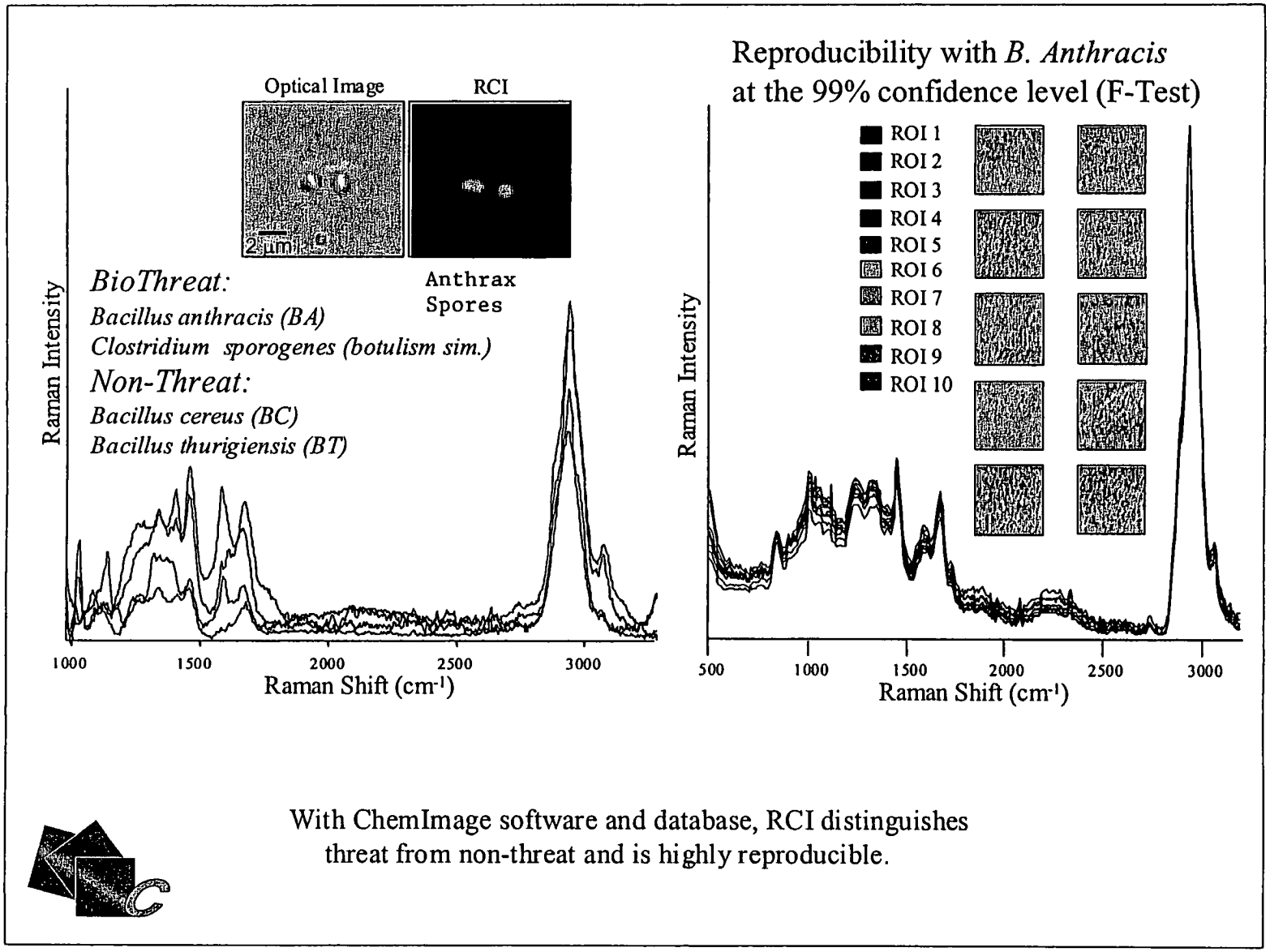






RCI reveals molecular-specific contrast in complex mixtures without the use of dyes or stains.

Figure 9



With ChemImage software and database, RCI distinguishes threat from non-threat and is highly reproducible.

Figure 10

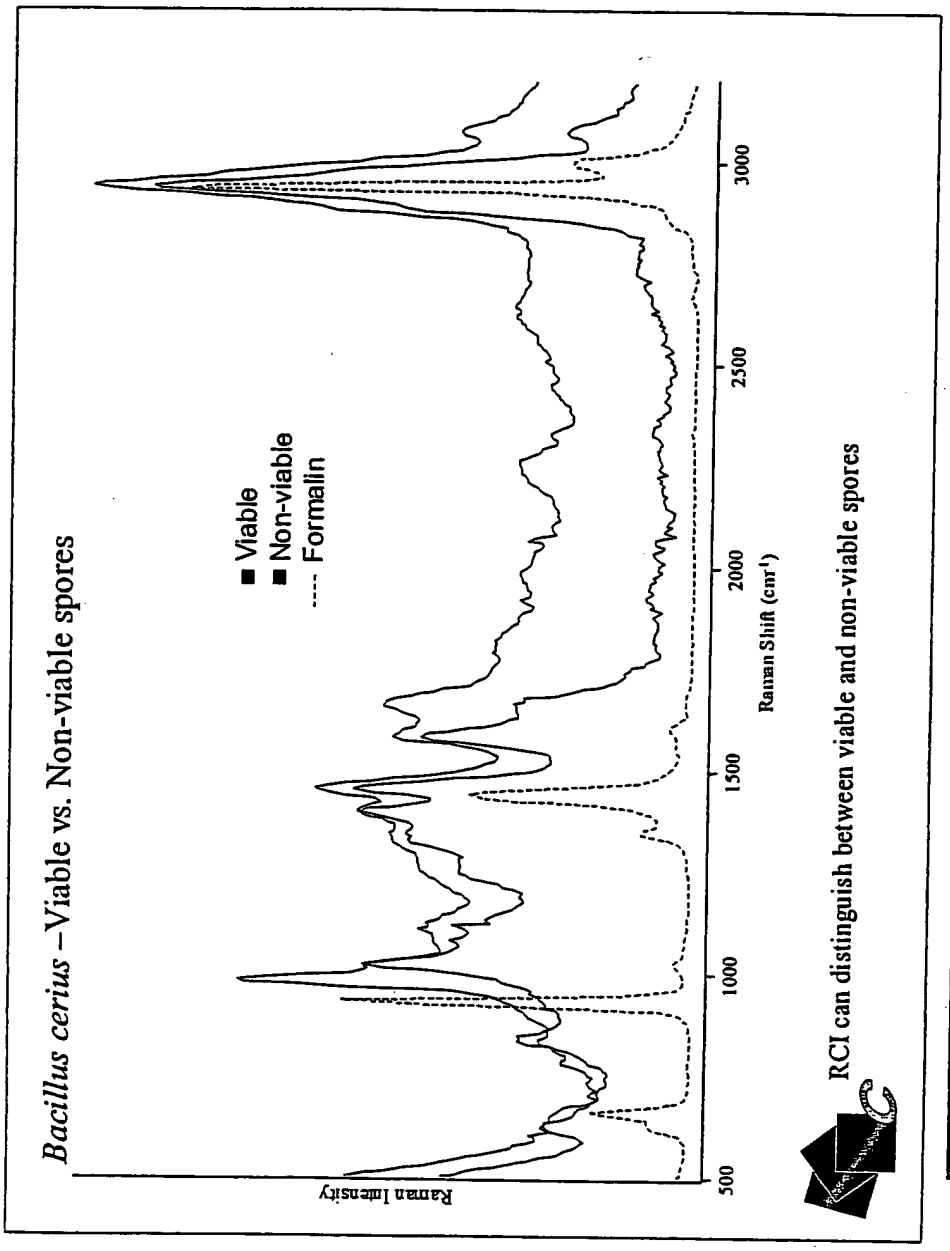

Bacillus cerius – Viable vs. Non-viable spores
Viable
Non-viable
Formalin
Raman Intensity
500
1000
1500
2000
2500
3000
Raman Shift (cm-1)
RCI can distinguish between viable and non-viable spores

Figure 11